NATURE

A Weekly Journal of Science

THE UNIVERSITY OF MICHIGAN

Vol. 187, No. 4741 SATURDAY, SEPTEMBER 10, 1960 2s. 6d.

OCT - 3 1960

UNDERGRADUATE LIBRARY

ZONE MELTING

The B.T.L. Zone Melting Apparatus, Semi-Micro Model is developed from the original N.C.L. model and handles quantities from 0.15g. up to about 2g. It can be used for refining organic compounds to a very high degree of accuracy, or for concentrating impurities in samples for analysis. Three interchangeable metal guide sleeves with heating elements are provided, to accommodate glass tubes of 12mm, 8mm and 5mm diameter, giving maximum temperatures of 210°C, 250°C and 310°C respectively. The apparatus is compact and easily transportable; overall size 9″x9″x15″ high.

Cat. No. C14/510.

A Macro Model handling quantities of the order of 1kg. is also available, Cat. No. C14/500.

Please send for full details of each model.

BTL complete laboratory service

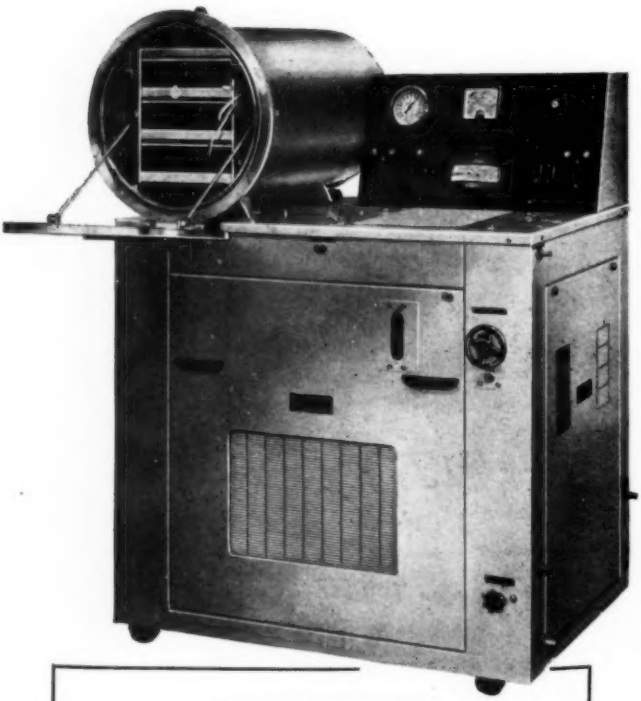
BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND.

Branches in London, Manchester & Glasgow. *Agents throughout U.K. and all over the world.*

"SPEEDIVAC"
30P1T

COMPLETE DRYING SERVICE FROM INITIAL FREEZING TO FINAL STOPPERING UNDER VACUUM

VACUUM FREEZE DRYING UNIT



The 30P1T is a compact mobile pilot plant for either research or small scale production. It is a shelf freeze drier with self-contained services and has been developed for drying or concentrating duties which require controlled conditions throughout the initial freezing and subsequent drying cycle.

This new unit provides the means for maximum exploitation of the freeze drying process for biochemicals, antibiotics, drugs, foodstuffs, etc. The shelves can be refrigerated or heated as required and can accommodate powder, cake or liquid containers.

★ Complete cycle from initial freezing to final stoppering can be carried out without breaking the vacuum in the chamber.

★ Shelves cooled to —20°C. by refrigeration or heated by thermostatically controlled heaters bonded beneath each shelf.

★ Condenser refrigerated to —50°C. by hermetically sealed compressor.

★ Complete instrumentation for measuring condenser and product temperatures and vacuum pressure in the chamber.

SPECIFICATION

Stainless steel drying chamber containing three shelves (approx. 10 in. by 24 in.) (25 × 60 cm.) providing a total shelf area of 4½ ft.² (4,500 cm.²). Shelves will accommodate approximately 750–25 mm. old flat bottom vials. A hermetically sealed refrigerator compressor serves the water vapour condenser and a separate refrigerator compressor provides cooling for the shelves by direct expansion. A "Speedivac" 2SC150 two stage rotary pump is incorporated in the pumping system and pressure in the drying chamber is measured by 71B thermal (Pirani) type vacuum gauge.

EDWARDS HIGH VACUUM LTD | MANOR ROYAL | CRAWLEY | SUSSEX | Crawley 1500

NATURE

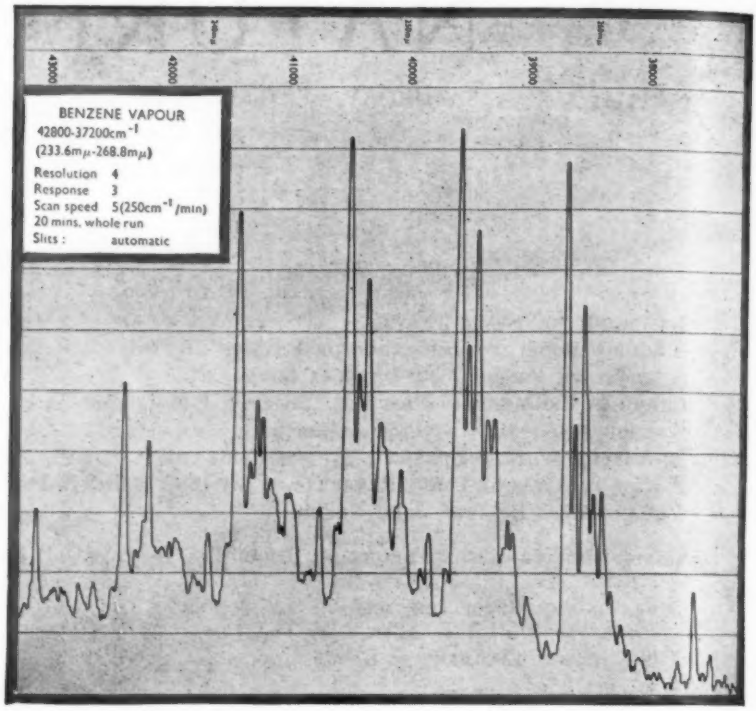
No. 4741 SATURDAY, SEPTEMBER 10, 1960 Vol. 187

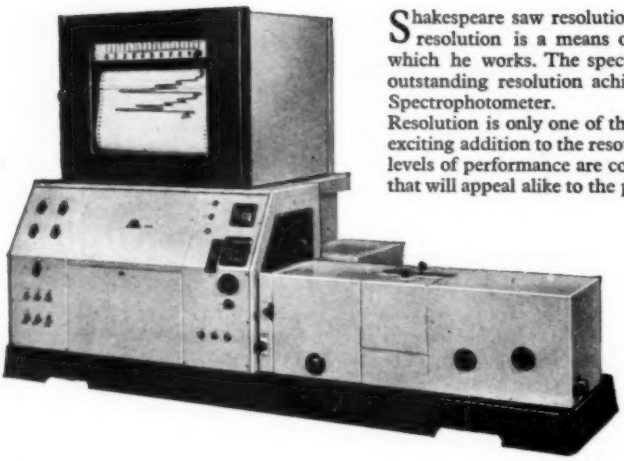
CONTENTS

	Page
The Need for Educational Research	883
Designing for Space Travel	884
A Source-Book of Semiconductor Physics. By Prof. G. F. J. Garlick	885
Solid State Physics. By Dr. R. O. Davies	886
Chemical Engineering Practice. By Prof. J. M. Coulson	887
Science and Meat. By Prof. J. Hawthorn	888
Biography of *Australopithecus*. By Mrs. Sonia Cole	888
A New Treatise on Plant Physiology. By Prof. Walter Stiles, F.R.S.	889
Vitamin B_{12}. By Prof. John Yudkin	889
Chromosomes and the Theory of Heredity. By Prof. C. D. Darlington, F.R.S.	892
Streaming in Cytoplasm dissociated from the Giant Amœba, *Chaos chaos*. By Prof. R. D. Allen, J. W. Cooledge and P. J. Hall	896
The National Engineering Laboratory, 1960. By R. I. Lithgow	899
News and Views	901
British Colonial Territories	906
Science and Education in Britain	906
Adult Education	907
The Mellon Institute	908
The National Institute for Research in Dairying. By Dr. J. A. B. Smith, C.B.E.	909
New American Observatories. By V. C. Reddish	910
Biological Effects of Exposure to Intense Radiation	910
Aviation Hygiene and Sanitation	911
Centenary Celebrations of W. M. Bayliss and J. S. Haldane in Moscow. By D. A. Sakharov	912
Is Hyperconjugation Necessary? By C. N. R. Rao	913
The 'GBR Experiment': a Trans-Atlantic Frequency Comparison between Cæsium-controlled Oscillators. By J. A. Pierce, G. M. R. Winkler and R. L. Corke	914
Chemical and Physical Properties of the Small Deoxyribonucleic Acid Component of Crystalline Cytochrome b_2. By Dr. M. D. Montague and Prof. R. K. Morton	916
Mechanism of Starch Biosynthesis. By Dr. Maria A. Rongine de Fekete, Dr. Luis F. Leloir and Dr. Carlos E. Cardini	918
Analysis of Metabolic Rates at the Cellular Level. By L. G. Lajtha, R. Oliver, R. J. Berry and Elizabeth Hell	919
Terminology in Plant- and Soil-Water Relations. By Dr. R. O. Slatyer and Prof. S. A. Taylor	922
A Genetically Determined Enzyme in the Pig. By Dr. K.-B. Augustinsson and Dr. B. Olsson	924

(*Continued overleaf*)

"How high a pitch his resolution soars"





Shakespeare saw resolution as a virtue in man: to the spectroscopist, resolution is a means of evaluating the quality of the tools with which he works. The spectrum of benzene vapour above shows the outstanding resolution achieved by the UNICAM SP.700 Recording Spectrophotometer.

Resolution is only one of the features that make the SP.700 a new and exciting addition to the resources of the analytical chemist. The highest levels of performance are combined with a speed and ease in operation that will appeal alike to the precise research worker and the busy works chemist. Great savings in time and cost go hand in hand with a major increase in both quality and quantity of analytical information.

Write to-day for a copy of the new, comprehensive specification of the Unicam SP.700 Recording Spectrophotometer.

UNICAM *Ultraviolet, visible and near infrared*

SP.700 Recording Spectrophotometer

UNICAM INSTRUMENTS LIMITED · ARBURY WORKS · CAMBRIDGE

U195

LETTERS TO THE EDITORS

Page

ASTROPHYSICS

The Solar Flare of June 1, 1960.—Prof. M. A. Ellison, Susan M. P. McKenna and J. H. Reid 926
Effect of Upper Atmosphere Wind on the Rotation of Satellite 1958δ1.—Dr. C. R. Faulkner . 926

RADIOPHYSICS

Possibility of detecting Ionospheric Drifts from the Occurrence of Spread *F* Echoes at Low Latitudes.—R. W. Knecht 927

PHYSICS

Thermal Expansion at Low Temperatures.—Guy K. White 927
Effect of Temperature on the Expansion on Setting of Gypsum Plaster.—P. J. Sereda . . 929
Packing of Mixtures of Hard Spheres.—Prof. Paul C. Mangelsdorf, jun., and Elmer L. Washington 930
Radioactive Labelling of Membrane Filters for the Assay of Air-borne Material.—Dr. G. Kubie and Dr. K. Spurný 930

RADIATION CHEMISTRY

Changes in Conductivity of Pure Water caused by X-Irradiation.—K. Schmidt . . . 931
Radiation-induced Nitration of Aliphatic Hydrocarbons.—Dr. Tyson Rigg 933
Role of Oxygen in the Cross-linking and Degradation of Deoxyribonucleic Acid by Ionizing Radiations.—Peter Alexander and J. T. Lett 933

CHEMISTRY

Possible Discovery of Ion-Pairs by Interferometry.—Prof. R. N. O'Brien and Mrs. C. Rosenfield 935
Solid–Solid Interactions on Active Adsorbents.—Dr. Philip Anthony and Prof. Harry Zeitlin . 936
Hydrocracking of Neopentane and Neohexane over Evaporated Metal Films.—Dr. J. R. Anderson and B. G. Baker 937

BIOCHEMISTRY

Assay of Corticosteroids in the Chick Embryo.—Sir Macfarlane Burnet, O.M., F.R.S., and N. L. Warner 939
Degradation of Benzylpenicillin at *p*H 7·5 to D-Benzylpenicilloic Acid.—Dr. Bernard B. Levine 939
Formation of D-Penicillamine-Cysteine Mixed Disulphide by Reaction of D-Benzylpenicilloic Acid with Cystine.—Dr. Bernard B. Levine . 940
New Inhibitor of Monoamine Oxidase.—Dr. J. D. Taylor, A. A. Wykes, Y. C. Gladish and W. B. Martin 941
Syntheses of Glucose, Cellobiose and Other Saccharides by *Acetobacter acetigenum* in a Lactate-buffered Glycerol Medium.—Dr. C. P. Jackson and K. Ramamurti 942
Distribution of Protein-bound Aminoazo Dyes in the Rat Liver Microsome.—Dr. T. Yamada, M. Matsumoto, S. Kanda and Prof. H. Terayama 943
Water-soluble, Non-fibrous Deoxyribonucleoprotein from Calf Thymus Nuclei.—Dr. Richard S. Welsh 943

PHYSIOLOGY

Adenosine Triphosphate and Maintenance of Shape of the Human Red Cells.—Makoto Nakao, Toshiko Nakao and Saburo Yamazoe . 945
An Abnormal Serum Protein in Experimental Poisoning by Cadmium and other Metals.—D. J. Lawford 946

Page

Comparison between the Effects of Cysteine and of Anoxia on the Rate of Mitosis in Lens Epithelium.—Dr. Antoinette Pirie and Dr. Ruth van Heyningen 947
Serotonin, a Melanocyte-stimulating Component in the Dorsal Skin Secretion of *Xenopus laevis*.—Dr. F. C. G. van de Veerdonk 948
Sodium and Potassium in Mitochondrial Membrane Fractions.—Dr. Frank Ulrich . . . 949
Antigonadotropic Function of the Pineal Gland and the Oxytocin of the Neurosecretory Hypothalamic System.—Prof. S.-M. Milcou and S. Pavel 950
Autonomic Nerve–Smooth Muscle Transmission.—G. Burnstock and Mollie E. Holman . . 951

PSYCHIATRY

Paradoxical Blocking and Arousal in the Drowsy State.—Dr. L. Goldie and Dr. J. M. Green . 952

RADIOBIOLOGY

Dye-sensitized Photo-reactivation of X-ray Damage in Diploid Yeast.—Dr. David Freifelder and Prof. Robert B. Uretz 953
Influence of Localized Ultra-violet Irradiation on the Incorporation of Adenine-8-^{14}C and D,L-Methionine-^{35}S in *Acetabularia mediterranea*.—Prof. J. Brachet and Dr. M. J. Olszewska . 954
Identification of Transplanted Bone Marrow Cells in Radiation Chimæras.—P. Czerski, O. Rosiek and J. Sabliński 955
Urinary Excretion of β-Amino-*iso*butyric Acid after Injection of Thymidine labelled with Tritium.—Dr. Georg B. Gerber, Gisela Gerber and Kurt I. Altman 956

BIOLOGY

Electrical Sensitivity of the Ampullæ of Lorenzini.—Dr. R. W. Murray 957
Development of Delayed-type Hypersensitivity in Guinea Pig Embryos.—Jonathan W. Uhr . 957
Insects attacking *Striga*.—D. V. Murthy . . 959
Age Determination of Seals.—H. R. Hewer . 959
Abnormal Crimping in Merino and Polwarth Wools.—R. E. Chapman, B. F. Short and P. G. Hyland 960
Banana Fruit-spot ('Speckle') caused by *Deightoniella torulosa* (Syd.) Ell.—Dr. D. S. Meredith . 961
A Method for the Isolation of Plant Protoplasts and Vacuoles.—Dr. E. C. Cocking . . . 962

ENTOMOLOGY

Bacteria as a Source of Food for Black-Fly Larvæ.—F. J. H. Fredeen 963
Accessory Glands of Gene's Organ in Ticks.—Dr. B. Feldman-Muhsam and Y. Havivi . . 964

CYTOLOGY

Induced Fluorescence with Acridine Orange, after Osmium Fixation.—W. J. Randles . . 964
A Polyploidizing Principle in the Spleen.—I. Bouvier, Prof. R. Rigler and W. Rosenkranz 965

SOIL SCIENCE

C Horizon of the Soil Profile.—Dr. S. G. Willimott and D. W. Gilchrist Shirlaw 966
Effect of Surface Density of Charge on the Physical Swelling of Lithium Montmorillonite.—Dr. J. P. Quirk and B. K. G. Theng . . . 967

MATHEMATICS

Theory of Optimal Games.—Prof. Alan S. C. Ross 968
A Remark on Stochastic Path Integrals.—Prof. M. S. Bartlett 968

DIFCO *microbiological reagents and media*

Bacto-Dehydrated Media for Microbiological Assay of
VITAMINS and AMINO ACIDS

These *media* contain all the necessary nutriments for the growth of specified test organisms for the microbiological assay of vitamins and amino acids except for the component under assay. These basal media require only the addition of specified increasing amounts of the vitamin or amino acid being assayed to obtain a growth response which may be measured by acidimetric or turbidimetric methods for the construction of standard curves. The vitamin or amino acid content of the material under assay is determined by adding appropriate concentrations of the test substance to the basal medium and comparing the growth response obtained with that of the standard.

BACTO-RIBOFLAVIN ASSAY MEDIUM
BACTO-NIACIN ASSAY MEDIUM
BACTO-THIAMIN ASSAY MEDIUM
BACTO-PANTOTHENATE ASSAY MEDIUM
BACTO-B_{12} ASSAY MEDIUM USP
BACTO-CS VITAMIN B_{12} AGAR
BACTO-FOLIC ACID ASSAY MEDIUM
BACTO-PYRIDOXINE ASSAY MEDIUM
BACTO-BIOTIN ASSAY MEDIUM
BACTO-CHOLINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
BACTO-LEUCINE ASSAY MEDIUM
BACTO-METHIONINE ASSAY MEDIUM
BACTO-LYSINE ASSAY MEDIUM
BACTO-ISOLEUCINE ASSAY MEDIUM
BACTO-ARGININE ASSAY MEDIUM
BACTO-TYROSINE ASSAY MEDIUM
BACTO-CYSTINE ASSAY MEDIUM

The *method* employed in carrying stock cultures of the test organisms and preparing the inoculum for microbiological assay is important. The following media have been developed especially for carrying stock cultures and for preparation of the inoculum:

BACTO-MICRO ASSAY CULTURE AGAR
BACTO-MICRO INOCULUM BROTH
BACTO-B_{12} CULTURE AGAR USP
BACTO-B_{12} INOCULUM BROTH USP
BACTO-NEUROSPORA CULTURE AGAR

BACTO-VITAMIN FREE CASAMINO ACIDS, dehydrated, is an acid hydrolysate of vitamin-free casein prepared especially for laboratories investigating microbiological assay of vitamins.

Over 60 years' experience assures UNIFORMITY · STABILITY · ECONOMY

Difco Laboratories

Prompt delivery from U.K. stock. Write for Difco Manual and technical leaflets to the sole agents:

BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND
Branches in London, Manchester and Glasgow *Agents throughout U.K. and all over the world*

TAS/DC4

Ultra-Violet & Infra-Red Sampling Instruments

ULTRA-VIOLET ABSORPTION CELLS
VARIABLE path-length cells for liquids
FIXED path-length cells for liquids

KBR EVACUABLE DIE
for making 13, 15 and 16 mm. Discs

INFRA-RED ABSORPTION CELLS
VARIABLE path-length cells for liquids
FIXED path-length demountable and sealed cells for liquids
MICROCELLS for liquids
GAS cells in pyrex and metal

HYDRAULIC 30-TON PRESS
especially designed for KBr Disc Technique

Rotating Electrode (Solution Excitation) Spark Unit

HIGH-SPEED VIBRATION MILL
indispensable for KBr Disc Technique
available for 230 volt 50-cycle and 110-volt 60-cycle mains

ROCK-SALT POLISHING KIT

RIIC Sampling Instruments are marketed by the leading spectrometer manufacturers of the world. Descriptive literature on application.

RESEARCH & INDUSTRIAL INSTRUMENTS CO.
116 Lordship Lane, London, S.E.22 Telephone: Forest Hill 2281/2

THE NEED FOR EDUCATIONAL RESEARCH

WHEN the first part of the Crowther Report was published in December 1959 it was recommended that more information about education should be obtained and made available : "... attention will have to be paid to the inadequacy of the tools that lie to the hand of the educational planner . . . There are the most extraordinary gaps in our knowledge of what goes on in the schools and technical colleges we have to-day . . . ". This is sharply brought out in Vol. 2 of the Crowther Committee's Report, which has now been issued*.

Before preparing its Report, the Committee was required to consider the work of technical colleges, the teenager in employment or industrial training, in the youth service and at leisure. The members always had to remember that two sexes were involved, each presenting its own educational and social problems ; and although little was known about the way educational and industrial and social pressures influenced boys, even less was known about their effects on girls.

What the Committee had not expected was that the factual evidence on which it could consider its terms of reference could be so meagre . . . "the information was sometimes lacking both to gauge the task or estimate the harvest. For instance, though an appreciable expansion in the output of students through the technical college courses was accepted as a major aim in public policy on which economic health on the long term depended, little was known nationally in 1956 about the circumstances in which students succeeded or, more often, failed, and how many years of study success or failure had cost them".

To help the Committee in its deliberations, special surveys were arranged with respect to the relation between home background and length of school life, of the progress of students through technical courses, the use of leisure, and the reserves of latent ability.

It is unfortunate that these three surveys could not have been published at the same time as the Report. They show both how slender was the evidence on which the Committee's sweeping recommendations were based and, more important, how much so-called educational progress is based upon popular—and sometimes passing—public opinion of a particular age.

In these surveys, for example, there is little or no evidence to support the Committee's major proposal to raise the school-leaving age for all children to sixteen. Accepting that "the attitude of the school-leavers themselves is obviously of importance in any examination of factors which may influence the length of school life", the investigators reported that "it was impossible to deal more than superficially with attitudes to the school-leaving age and reasons for leaving school". If any conclusion can be drawn from the survey report it is the rashness of the Crowther Committee in suggesting that *all* children should be kept in school until they are sixteen years of age. Thus, of the secondary modern boys and girls who had fairly recently left school and who were asked if they would have liked to stay longer, only 16 per cent of the boys and 23 per cent of the girls had regretted leaving school when they did.

A similar question was put to a group of National Servicemen, and despite their greater maturity and time for reflexion, only about one man in six who had left school at fifteen had afterwards wished that he had not left so early. Of those who had wished that they had remained in school longer, the investigators report that four-fifths of them realize that now, with better education, their chances of getting better jobs would be considerably improved. The investigators did not determine whether these young men of eighteen were seeking to remedy the defects in their education or whether they had the capacity to profit by it. Their wistful desire for more education may have been more bound up with the fruits of better jobs than with the effort required to attain them.

The Government's decision to make no firm forecasts about the date on which the school-leaving age is to be raised was largely based on financial and economic arguments. Before a great step of this nature is taken, it could well spend a little money to dig deep for information which has only been scratched in these surveys. Many well-informed people believe that it is educationally and morally wrong to compel all young adolescents to continue a formal education which many of them find irksome and restrictive, and which might well contribute less to their social and educational development than would a planned period of vocational training in industry supported by continued education of both a vocational and recreational nature.

If educational reformers are genuine in their desire to bring the greatest educational good to the greatest number, they should first accept that more than their own wishes and experience is needed before advocating a 'reform' which could well bring misery and frustration to many young people. Evidence has been accumulating that young people (and especially girls) of fifteen to-day are as mature as young people of sixteen and seventeen of 25 years ago. In terms of biological and psychological age, may not the 1944 goal of raising the school-leaving age to sixteen have already been reached ? More information about the wishes and feelings of young people is required.

For a number of years much has been made about the ill-effects of too much money on young wage-earners. Little real investigation has been carried out to determine the truth of this charge. The majority of young delinquents come from boys and girls still at school. Why ? Which types of schools—grammar, or modern, or comprehensive ? Also, from which classes

* Report of the Central Advisory Council for Education—England, 2 (Surveys). (H.M. Stationery Office.) 8*s*. 6*d*.

in the school? *A* stream or *D* stream? Is there direct correlation between the delinquents and the types of homes from which they come? Doctors, youth leaders and industrial training officers could well join parents and teachers in providing much useful information about the needs of young adolescents. So, too, will the investigation being carried out by the University of Sheffield into transition between school and work. Before the precedent is set of compelling all young people to remain at school until they are sixteen—at present those who stay on at grammar, technical or modern school do so because they wish—much educational research is needed into the biological and psychological and social requirements of young people and how they can best be met.

The case for more research to support educational thinking is also brought out in the third survey conducted on behalf of the Crowther Committee. This was undertaken to establish the broad facts about the efficiency of education continued by part-time day release classes. For some years investigators whose contacts with industry have been superficial have raised considerable dust about the wastefulness of City and Guilds and National Certificate courses. Their standards were set almost entirely by the number of students who began courses and the numbers who obtained Final City and Guilds and Higher National Certificates. (See also the article by Dr. J. Heywood in *Nature* of July 16, p. 189.)

The survey now shows that since National Certificate courses were begun in 1923 some 18,028 Ordinary National Certificates have been awarded, about a third of these going to students who left school at the minimum statutory age. "Neither should the wastefulness of the process be exaggerated. The shedding has taken place pretty steadily along the route, and it is not a fact that all those who have not reached the end of the road have wasted many hundreds of hours of their own and their teacher's time in fruitless endeavour." Those who have regarded the process as utterly wasteful have insisted that more attention be paid to selection, and that teachers in technical institutions should join industrial management in making the selection of industrial apprentices and others. This report makes it clear that much more than stringent selection at the outset is required. "The course itself is a kind of selective process, and all that may be hoped of a more stringent and deliberate process is that it would be somewhat, but not a great deal more efficient."

This report will remind many educationists of the Education Act of 1944. Against the advice of almost every educational psychologist, a tripartite system of secondary education was set up with rigid selection at the age of eleven. Although administratively convenient, the folly of the tripartite system has become only too evident in the last fifteen years. It is to be hoped that future reforms will be based much more on facts patiently gathered by careful investigation rather than by well-meaning intentions to do good. It is time the social reformer was replaced by the social engineer, who uses facts as a basis for change.

DESIGNING FOR SPACE TRAVEL

Soviet Space Science

By Ari Shternfeld. Translated by the United States Air Force (Technical Documents Liaison Office). Pp. xxii+361. (London: Hutchinson and Co. (Publishers), Ltd., 1959.) 30*s*. net.

Space Technology

Edited by Prof. Howard S. Seifert. Pp. xvi+1160. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 180*s*. net.

PERHAPS the most dramatic engineering development of the past few years has been the demonstration of the practicability of flights in the solar system at least by unmanned vehicles. The development of this technique has received a substantial impetus from motives as diverse as a recognition of a new, powerful tool for astronomical observations and the desire for pre-eminence in human space-travel for political reasons. Space technology is in consequence rapidly building up a literature to which the two volumes forming the subject of this review represent substantial additions. Since ultimately they emanate one from each of the two nations most concerned with these developments it might be supposed that they would provide an interesting comparison, but a comparison is vitiated by the fact that they aim at quite different readers. Moreover, "Soviet Space Science" draws information from sources on a wide international basis showing, if anything, a bias towards American work in the field.

The title is, in fact, altogether a misnomer, for this book is really concerned with space flight. The first and largest section, comprising five chapters, deals with the launching and subsequent motion of an artificial Earth satellite. The explanation is clear, detailed and non-mathematical, and at times unduly repetitive. It is evidently aimed at a non-technical reader since extensive use is made of tables and diagrams rather than graphs. Subsequent chapters are concerned with the hazards and physiological problems of manned space flight, the scientific uses of satellites and space probes, the problems of telemetry and communication, and landing. In these sections the author is content to skim over the surface of his subject; it is noticeable that no effort is made here, unlike the opening chapters, to expound the basic principles. Moreover, few references are made to the direction of future technological advances, even where their direction can be clearly discovered.

Particular exception can be taken to the account of experimental space science, which is very shallow, and where the language employed is often imprecise. (For example, the expression "the voltage of the current" is used.) There is not a large number of misprints in the book, and it is unfortunate that they should largely occur among the numerics in this section, where they are particularly liable to mislead.

There are two appendixes, dealing respectively with astronautical societies and the international law of space, an epilogue by Willy Ley, which brings the account of vehicles launched up to *Lunik* I and a good index.

"Space Technology", which deals primarily with the engineering of guided rockets for orbital missions, is based on a series of lectures delivered during 1958 at the University of California by a total of 38 different lecturers. The editor, Prof. Howard S. Seifert, has handled with considerable success the

problem of reducing to a reasonably uniform style and level contributions from such a large number of authors.

The book opens with a brief introduction of a historical and astronomical character, which is followed by an extensive account of the motion of powered and unpowered vehicles in gravitational fields. Approximate methods of calculation, such as perturbation techniques, are described for the many-body problems which are encountered, leading to the solution of design optimization problems. The topics covered in this way include ballistic missile trajectories, Earth satellite orbits and lunar and interplanetary flights (in which propulsion is used only near the beginning and end of the flight). These are followed by an account of the use of low-thrust motors, such as those employing positive-ion jet or nuclear fuels, to expand Earth satellite orbits for interplanetary travel. After a chapter on relativistic time dilatation effects, this section concludes with a description of methods of soft landing. It will be seen that this part of the book is both detailed and comprehensive. The explanation of some of the most complex orbital problems, such as the motion of a satellite in the Earth–Moon system, is excellent and brings out very clearly the principles involved. Similarly the limitations of various propulsion systems for different missions are thoroughly covered.

The subsequent sections are somewhat less detailed and deal with propulsion (using both chemical and nuclear fuels), rocket system design procedures, communications, guidance, and the physiological aspects of manned flight. The last section is rather briefly concerned with the upper atmosphere and the scientific uses of satellites. Finally, there is, even for the enthusiastic reader, an examination paper of 102 questions!

In conclusion, it can be said that "Space Technology" gives an excellent account of the methods employed to select optimum orbits. The description of motors and guidance systems provides a good background to this subject and an introduction to its technological aspects. A certain amount of information on the environment which will be encountered, in planetary atmospheres, for example, and on the performance which can be expected from human beings in a space ship, is also presented. Most of this is rather general in character, and these sections suffer from a rather low density of hard facts.

In perspective, both the books which are the subject of this review can be seen as descriptions, at quite different levels, of the dynamics of space vehicles together with an account which is given in breadth rather than depth of the associated technology and planetary physics. Each in its way can be recommended.

A SOURCE-BOOK OF SEMICONDUCTOR PHYSICS

Semiconductors

By Dr. R. A. Smith. Pp. xvii+494. (Cambridge: At the University Press, 1959.) 65*s*. net.

SEMICONDUCTOR physics has become a vast field of research and development over the past decade. Any text on the subject must of necessity be selective, but in the present volume the author has presented those topics which are of fundamental interest and moreover has provided critical discussion at all stages. This is particularly important for those who may use the book as an introduction to the theoretical aspects of phenomena with which they are concerned experimentally. It is perhaps not often realized that a semiconductor cannot always be distinguished by a negative temperature coefficient of resistance, as is pointed out in the first chapter. Here can be found a reference to other problems of early work in the field which were only resolved by the production of single crystal specimens of high purity, by the careful distinction between volume and surface effect on physical properties, and by recognition of the decisive part played by crystal defects and impurities. In introducing the essential wave-mechanical basis of semiconductor physics, Dr. Smith is careful to carry the reader forward to more complex configurations than simple spherical energy-surfaces in wave vector-space, and to indicate the important experimental approach obtained in cyclotron resonance. In treating crystal binding some brevity has obviously been necessary, but one cannot help wishing that the problem of mixed binding and experimental methods of assessment had been afforded more discussion. In reading the section on excitons the reader must not forget that in the case of some high-band-gap materials, such as cadmium sulphide, the existence of excitons is not satisfactorily proved.

Many of the phenomena associated with semiconductors occur under conditions of thermal equilibrium or with very small departures from it. It is not therefore surprising that a significant fraction of the text is devoted to energy distributions of electron and positive holes, to transport phenomena and to the very important matter of scattering mechanisms for carriers. This part of the book is very up to date; for example, a discussion of recent work on 'hot' electrons, that is to say, carrier behaviour in high electric fields, is included. There is also a very adequate treatment of thermomagnetic effects not always found in texts on solid-state physics.

Much information on semiconductors results from a study of optical properties such as the position and structure of the lattice absorption edge. The author gives a review of recent experiments and their significance in distinguishing between direct and indirect, phonon-assisted, electron transitions. Here he draws on powerful data obtained by his own research team, among others. Within the electro-optical phenomena discussed none is perhaps more important than the release of carriers by photon absorption, which manifests itself in photoconductivity, the photovoltaic effect and the photo-electromagnetic effect. These are described briefly at first, but elaborated later in the book when a treatment of carrier diffusion has been given which provides a more complete and essential background for discussion. In the latter section we find the p-n junction, the problems of contact and surface conditions and the mechanisms of carrier injection and recombination.

For many experimentalists it will be a pleasure to find that after many pages of theoretical treatment Dr. Smith anchors it all by a critical review of the experimental methods available for the determination of the many parameters which have previously emerged in the theory. His chapter on such methods effectively introduces the survey of practical semiconductors of various types, particularly germanium, silicon, intermetallic compounds and, from his

particular interests in the past, the sulphide, selenide and telluride of lead. The latter is a useful inclusion, although a more complete account has been given previously by the author and some of his co-workers. High-band-gap semiconductors may appear to have been neglected, but data for them are so incomplete and still inconclusive compared with those for such solids as germanium and silicon.

This book makes an important and critical contribution to the subject which is based on the author's personal experience as leader of one of the most important research teams of to-day in semiconductor physics. It will be an excellent 'source-book' for many workers in this and closely related fields, and will certainly satisfy an important need in postgraduate university courses of solid state physics.

G. F. J. Garlick

SOLID STATE PHYSICS

Solid State Physics

Advances in Research and Applications.
Vol. 7. Pp. xiv+525; 1958. 12 dollars.
Vol. 8. Pp. xiv+519; 1959. 13.50 dollars.
Vol. 9. Pp. xv+548; 1959. 14.50 dollars.
Vol. 10. Pp. xv+516; 1960. 14.50 dollars. Series edited by Frederick Seitz and David Turnbull. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd.)

Paramagnetic Resonance in Solids

By William Low. (Solid State Physics: Advances in Research and Applications, Supplement 2.) Pp. viii+212. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 7.50 dollars.

THE contributions to the volumes of "Solid State Physics" under review maintain the high standards of scholarship and readability set by previous writers in the series. Research workers in the field have come to rely so much on "Solid State Physics", both as an invaluable source for their own work and as a kind of lifebuoy to keep them afloat in the flood waters of the literature, that one wonders how the subject maintained any kind of unity before the series was started. Perhaps it did not; and perhaps even now individuals read only articles bearing directly on their tenth (or hundredth) of the field. If this is so, the best service a reviewer can do is indicate the nature of the topics discussed.

There are three major articles on electrons in metals. J. Callaway, in "Electron Energy Bands in Solids" (Volume 7), describes first the general properties of bands (with special reference to symmetry and the use of critical points) and then gives a complete survey of existing experimental and theoretical results. It is all very carefully done and it is apparent that the calculations are now sufficiently sound for experiments to constitute a genuine test of theoretical assumptions about core potentials. The oscillatory dependence of magnetic susceptibility and electrical conductivity on the magnetic field is considered by A. H. Kahn and H. P. R. Frederikse in Volume 9. These phenomena are now very effective tools for investigating electron bands—especially the shape of the Fermi surface. There is a fine, 200-page article by the late M. R. Schafroth on superconductivity in Volume 10. Half the article is phenomenological and half is microscopic; it constitutes the best available account of the exciting progress in this field since 1950. The author's exposition of Bogoliubov's version of the theory is specially lucid and convincing. In spite of the present inability to explain the vanishing resistance (which gives the phenomenon its name) and the still controversial explanation of the Meissner effect, the other phenomena are now so well explained that one must surely accept Schafroth's conclusion: we are very close to a final theory of superconductivity.

The next group of articles is concerned with lattice properties of solids. A. F. Wells, with characteristic clarity and excellent illustrations, describes and classifies inorganic crystal structures in Volume 7. This is done not from the formal basis of symmetry, but rather from a more friendly and intuitive 'topological' point of view which asks how crystals can be built up from given elementary units of bonds and nets. Also in Volume 7 is a long and useful review by H. B. Huntington giving theory, experimental methods and results concerning the elastic constants of crystals. Among many other topics, the author discusses Laval's question: "Should local rotations and torques be considered in forming the elastic tensor?" The verdict is 'not proven'. D. Lazarus in Volume 10 gives a full and satisfying account of diffusion in metals. In Volume 7, P. G. Klemens contributes an article on the difficult subject of lattice thermal conductivity. An attractive feature of the paper is the way in which the author describes how the measurement of thermal conductivity can be used, at least as a supporting technique, in the study of crystal defects: dislocations, stacking faults and grain boundaries.

Defects and inhomogeneities in crystals are discussed in three further papers. There is a long article on the structure and properties of grain boundaries by S. Amelinckx and W. Dekeyser in Volume 8. This succeeds well in tying together the modern theoretical work which treats grain boundaries as arrays of dislocations and the extensive experimental findings, structural and kinetic. Certain aspects of the continuum theory of stationary dislocations are developed further by R. de Wit in Volume 10 than they were taken by Eshelby in an earlier volume of "Solid State Physics". In curious contrast to the state of affairs 100 years ago, it seems that one now discusses elasticity with the help of electromagnetism. A. Guinier writes in Volume 9 on "Heterogeneities in Solid Solution". The article naturally deals with the Guinier–Preston precipitation-zones in aluminium–copper alloys, but includes much more, such as a section on short-range order in solid solutions in equilibrium.

The editorial policy of providing detailed surveys of important groups of semiconductors is continued here with three contributions. W. W. Scanlon writes in Volume 9 on a little-known group of polar semiconductors: lead sulphide, lead selenide and lead telluride. The important feature of these materials is that whereas they enjoy NaCl-type binding and structure, their energy gap is sufficiently small to allow the electrical properties to be investigated easily. They thus form more useful test materials for the theorist than alkali halides. A detailed account of electronic processes in zinc oxide is provided by G. Heiland, E. Mollwo and F. Stöckmann in Volume 8, and in the same volume R. Newman and W. W. Tyler review photoconductivity in germanium. The latter authors emphasize the

usefulness of germanium—the intrinsic properties and impurity levels of which are well understood—as a model for theories of photoconductivity. Consequently their article is much more than a collection of results.

In Volume 10, B. S. Gourary and F. J. Adrian present an elaborate account of the work on wave functions for electron-excess colour centres in alkali halides. This is one of four articles on the optical spectroscopy of solids. Two of them are on the relatively new field of molecular spectroscopy in molecular crystals (for example, benzene, anthracene) and cover virtually the same ground. I found the article by H. C. Wolf in Volume 9 more coherent and enlightening and indeed more up to date than the article on the same topic by D. S. McLure (Volume 8). However, the latter author has provided us with an excellent account of the spectra of ions in crystals (Volume 9), where, of course, the symmetry properties of crystalline fields are of vital importance.

The articles mentioned above contribute to well-established branches of solid state physics. Rather more exciting to the novelty seeker are a group of three reviews describing applications of the techniques of nuclear physics to problems of the solid state. Perhaps the best known of these topics is the use of thermal neutrons to investigate vibrational spectra, discussed in workman-like manner by L. S. Kothari and K. S. Singwi (Volume 8). More esoteric is the use of angular correlation measurements on oriented nuclei to get extra-nuclear data, examined by E. Heer and T. B. Novey in Volume 9. The idea is that in the time between successive nuclear emissions, local nuclear motion may perturb the intermediate nucleus. The information obtained is similar to that found by studying spin-lattice interactions by nuclear magnetic resonance. Although the technique is clearly far less flexible and general than that of nuclear magnetic resonance, it may be useful in some cases because, first, no radio-frequency field is needed and, secondly, it can be made to work with about 10^8 nuclei cm.$^{-3}$ as compared with the nuclear magnetic resonance requirement of 10^{18} nuclei cm.$^{-3}$. Perhaps the main lesson to be learnt from D. R. Wallace's account of position annihilation in solids and liquids (Volume 10) is that this phenomenon is less a tool than a challenging problem in its own right—one that is far from exhausted.

New results on surfaces are expounded by J. A. Becker in Volume 7. They have been obtained by the ingenious use of ion gauges and field emission microscopy. It is remarkable that Müller's recent extension of the latter technique, in which ions are used instead of electrons to form the image, permits surface pictures which, at least in some respects, show the surface on an atomic scale.

I was specially attracted by a delightful article by D. J. Montgomery (Volume 9) on one of the oldest, yet least understood, topics in physics: the static electrification of solids. While concealing none of the essential complexity of the problem the author has been bold enough to describe a simple rational scheme based on electron transfer from the bulk of the solid (thereby ignoring specific surface effects) which seems to give a 'zero order' account of the main phenomena. Teachers who dabble in the catskin and amber branch of witchcraft will read this article with pleasure and profit.

In addition to the main volumes described above, there is a second 'supplement' consisting of a monograph on paramagnetic resonance in solids by W. Low. Although most of the material can be found in three well-known reviews published elsewhere, this is the first connected account of the whole subject since the early 1950's. It is admirably done, and will clearly become a theoretical handbook for workers in the field. R. O. Davies

CHEMICAL ENGINEERING PRACTICE

Chemical Engineering Practice
Edited by Herbert W. Cremer and Sidney B. Watkins. Vol. 11: Works Design, etc. Pp. vi+390+xviii. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1959.) 95*s.*; 13·30 dollars.

CHEMICAL works are frequently large in area, and in their general design and administration present problems which are to be found in all large enterprises. In this volume we are taken away from the specialized field of chemical plant design and are concerned with the more general topic of works construction and organization; we are concerned with the factory and its problems rather than with the details of any particular unit.

This is a guide to the structure of a large chemical works. We are brought face to face with the problems of management of people, of the arrangement of contracts, and with planning and production and its impact on sales. The peculiarities of the chemical industry, with its frequent demand for continuous working and the resulting problems for personnel, its hazards arising from the toxic and inflammable nature of many of its materials and processes—all these difficulties are clearly indicated as part of the life of a chemical works. In the very long section on works administration Mr. Hiscock of the Consolidated Zinc Corporation, Ltd., gives some suggestions for their solution. Those entering these industries are usually crammed with academic facts, but here they can see that they will be brought problems which will demand an entirely new approach. It is not simply a question of learning the techniques of a given industry, it is a question of harmonizing the demands of a very wide range of people to form a happy community. There must be an understanding of what constitutes incentive. "Work study does not replace management, nor does it necessarily ease the burden of management". "Method study presents facts; it does not make the decision. Whether to proceed is the responsibility of management". The importance and problems of work study in the chemical industry are in general agreed; but it is very useful to have the facts set out, and their limitations, as in this text.

The chemical industry in Britain, like other enterprises, is governed by Factory Acts and other legislation. Many in all positions in the chemical industry will be indebted to Sir George Barnett for his most welcome chapter on "Factory Acts and Legislation". This chapter, together with that by Dr. Carter on "The Alkali Acts and Works Regulations", should be read by all who contemplate entering this industry. We can see from this text the kind of organization that must be developed if the problems of the chemical industry are to be tackled and inducements given for profitable working in the company. This is above all a record of experience, of personal observa-

tion and analysis. We can learn from here something of the secret of 'what makes a factory tick'. We can learn from a body of men who have all taken an active part in developing industries of this kind. This is an excellent volume for which we should be grateful to Mr. Cremer and Mr. Watkins.

J. M. Coulson

SCIENCE AND MEAT

The Science of Meat and Meat Products

By Members of the Research Staff of the American Meat Institute Foundation. (Series of Books in Agricultural Science.) Pp. x+438. (San Francisco and London: W. H. Freeman and Company, 1960.) 68*s*.

THE growth of education in the subject which has come to be known as food science has been handicapped by a lack of suitable texts. For this reason the publication of "The Science of Meat and Meat Products" is an event of some importance. Yet the book is more than a text. As its preface claims, it is no less than an attempt "to present the current scientific knowledge on meat and meat products, and to show how various scientific disciplines are applied in meat research."

In common with other foodstuffs, meat has attracted the attention of chemists, biochemists, nutritionists, microbiologists and others for more than half a century. The quickening tempo of research in recent years has both stimulated and been stimulated by the increasing industrial demand for fundamental information. The problem starts with the geneticist in breeding from the gene pools at his disposal the types of animal best suited for the varied purposes of use. It ends perhaps at the level of human nutrition; but between the beginning and the end diverse scientific disciplines have many parts to play.

With this field to survey within the compass of a modest 438 pages, the American Meat Institute Foundation has undertaken a substantial task. It is inevitable that an individual reader will find some topics treated less fully than he would have liked. Moreover, the various chapters were written by a panel of twenty-six authors, and some unevenness of treatment would seem inescapable. That the reader is not unduly conscious of multiple authorship in the circumstances is a tribute to the editorial board. Formal references have been wisely avoided, but at the end of each chapter a carefully selected list of source material is given to ease the path of those who would study further. The compression of factual information does not always make for easy reading, but the style throughout is lucid, and comprehension is aided by excellent illustrations.

The necessity for keeping the book in proportion has cramped the writers of certain sections, and it is to be regretted that space has been devoted to topics such as Michaelis-Menten kinetics and the methods of protein chemistry which will be familiar to most of its readers. The space saved by omitting such material might have been devoted to the anatomy of meat animals and to a fuller treatment of the relations between carcase conformation and meat quality. The average butcher is a better anatomist than some scientists engaged in the study of meat.

The indexing of such a book presents peculiar problems, and the index, although good, is not comprehensive and fails to list some of the important items considered in the text. Few misprints were noticed, apart from a very obvious one in the table of contents, but a curious mistake in connexion with federal regulations governing the use of ascorbic acid was noted on p. 359.

Notwithstanding these criticisms, this book is an important work which may become a standard text. It is an essential purchase for food science libraries, and a laboratory copy in British meat-packing plants might well contribute to improvements in practice in our own industry.

J. Hawthorn

BIOGRAPHY OF *AUSTRALOPITHECUS*

Adventures with the Missing Link

By Prof. Raymond A. Dart, with Dennis Craig. Pp. xv+251+20 plates. (London: Hamish Hamilton, Ltd., 1959.) 25*s*. net.

PROF. Dart has brought together many of his controversial theories in this book, which is partly autobiographical, but mainly a biography of *Australopithecus*. The story begins with the discovery of the Taungs infant in 1924 and ends with a footnote on *Zinjanthropus* added while the book was in the press.

Nearly half the pages concern the 'osteodontokeratic culture', which has been Dart's main interest for 14 years. Only by proving that the Australopithecines were tool-makers could he resolve the interminable arguments as to their hominid or pongid status. Sceptics discredited the 'culture' as the work of bone-collecting hyenas, a myth which Dart took great trouble to disprove. His detailed analysis of bone fragments from Makapansgat revealed that the great majority were antelope mandibles and the distal ends of humeri. By comparing these finds with a similar bone 'industry' from a Middle Stone Age site at Kalkbank, he became convinced that the Makapansgat specimens were artefacts.

Whether one agrees with Dart's conclusions or not, he cannot be accused of drawing on too little evidence. The task of sorting thousands of tons of breccia, chiselling out the bone fragments and identifying them must have been formidable. Nor did he neglect practical experiments, such as reproducing the famous cannon bone 'apple-corers' which necessitated striking over a hundred blows with a bone fabricator. But to suggest that the Australopithecines went to such lengths to make scoops to convey pulped food to the mouths of toothless infants is surely to overestimate their technological skill—and our own credulity.

Dart's genius and persistence might be interpreted by some as impetuosity and obduracy. He makes no attempt to conceal his faults, of which the most serious is touchiness. He refused to study the Mapungubwe skeletons because "any report I might make on the remains would be regarded as prejudiced" (p. 68); and would not interpret the endocranial casts from Sterkfontein because of Hooton's remark that "a dispassionate interpretation of new fossil evidence is usually obtainable only . . . by persons not emotionally identified with the specimen" (p. 86).

Some of the causes which Dart took up with such enthusiasm found few supporters: the Eastern origin of Rhodesian mining and stone building; Mongoloid contacts with the Bushmen; the fire-making abilities

of *Australopithecus prometheus*. But on several occasions he has been proved triumphantly right, notably over the tool-making capabilities of the Australopithecines—in stone. His unorthodox views and his imagination, no less than his discoveries, have had a most stimulating effect on anthropology. They are very apparent in this most absorbing and readable book, which is marred only by the poor quality of the drawings.

SONIA COLE

A NEW TREATISE ON PLANT PHYSIOLOGY

Plant Physiology

A Treatise. Edited by F. C. Steward. Vol. 2: Plants in Relation to Water and Solutes. Pp. xvii+758. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1959.) 22 dollars.

PLANT physiologists cannot complain of a shortage of literature designed to provide up-to-date information of the state of knowledge of the various branches of the subject. As well as a number of monographs on different parts of the subject there has appeared each year for the past ten years the "Annual Review of Plant Physiology" which has given summaries of recent advances in selected aspects of plant physiology, while about four years ago the publication of the "Encyclopedia of Plant Physiology" began, about a third of its projected 18 volumes having still to appear. Now we have the first volume of what is described as a treatise which is designed to cover the field of plant physiology in seven volumes. Although very much smaller in size than the "Encyclopedia", this new venture will yet be a considerable work when completed. How then is it to be appraised? According to the preface of this first volume to appear, it is intended not only to be a work of reference but also to give "a reasoned analysis of the status and development" of each subject discussed, and the readers for whom this analysis is intended are listed as "advanced and postgraduate students, teachers, research workers and investigators in other fields of knowledge, who need information about the present state of physiology".

This first volume to appear consists of seven articles, varying very much in length, by writers who have worked in the respective branches of the subject with which they deal. As is pointed out in the preface, each of these articles is affected by its author's own opinions. This should be borne in mind by the reader, for other workers in these same fields might have other opinions. For example the reviewer, who has had more than a casual interest in the salt relations of plant cells and tissues for nearly half a century, and who was concerned in some degree with earlier investigations on these relations, also has his own opinions, and it is clear that these do not always coincide with those expressed in the article on this subject in the volume under consideration.

Works of this kind, well produced and well bound, extending to several volumes and composed of a greater or less number of articles written by different contributors, have become the fashion. Of their value to the student there can be no doubt, but whatever their merits they have one character in common: they cost what seems to the reviewer quite a lot of money. This is no doubt unavoidable, but it probably means that in Great Britain at any rate they find their way mainly into the libraries of universities and other institutions and that comparatively few are purchased by students and teachers, who are not among the most opulent members of the community. If this should be so with the present volume it is to be regretted, as probably many plant physiologists would like to have it on their own shelves

WALTER STILES

VITAMIN B_{12}

Vitamin B_{12}

By Dr. E. Lester Smith. (Methuen's Monographs on Biochemical Subjects.) Pp. xii+196. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1960.) 15*s*. net.

VITAMIN B_{12} is unique in many ways. It took more than twenty years from the time of the discovery in 1926 that liver cured pernicious anæmia until the isolation in 1948 of the active principle, which we now know as vitamin B_{12}. The vitamin is active in fantastically small quantities; the body needs something of the order of one millionth of a gram daily. This is about one thousandth of the requirements of some of the other vitamins in the B groups. Vitamin B_{12} is the only vitamin which contains a metal in its very complicated molecule. It seems to be synthesized only by micro-organisms, yet in our diets it exists virtually exclusively in foods of animal origin. Its final isolation in 1948, almost simultaneously in the United States and in England, would no doubt have been even longer delayed but for the resources of the two large pharmaceutical companies which could work up one ton of liver to produce about 20 mgm. of red crystals.

Dr. Lester Smith, the Glaxo chemist who was responsible for the isolation in England, has produced the first book devoted entirely to vitamin B_{12}. It is a short book, and this is probably the reason for the only serious criticism one can make of an otherwise excellent survey. Some of it is so condensed as to make it difficult to understand at first reading; it becomes more of a reference book than a simple account of the subject. Of particular usefulness for reference is the description of the chemistry of vitamin B_{12} and its many relations; this is splendidly done by the author, who himself has contributed a good deal to its elucidation. On the other hand, a few errors and ambiguities—none of them at all serious—have crept into the description of the biological aspects of the vitamin. For example, the author cites the work of Morgan and Yudkin on the vitamin B sparing action of sorbitol, but this work in fact makes no reference to vitamin B_{12}. Again, how many readers know of the enzyme rhodanese? I have only one criticism of production: it is certainly difficult to print chemical formulæ so as to give the effect of three dimensions, but something better can be done than those given on pp. 36–37.

This is a book that every nutritionist will have to possess and use; and we shall look forward to new editions produced by Dr. Lester Smith to keep us up to date in the rapidly advancing knowledge in this field. I hope he will then be kind enough to indulge the prejudices of at least one reader, who believes that 'human' is an adjective and not a noun, and who also believes that there is a difference between the symptoms and the signs of an illness.

JOHN YUDKIN

Advances in Clinical Chemistry

Vol. 2. Edited by Harry Sobotka and C. P. Stewart. Pp. xiii+387. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1959.) 12 dollars.

TWO of the articles in this volume are primarily concerned with methods ("Paper Electrophoresis" by H. Peeters and "Automation" by W. H. Marsh). The four others survey certain selected biochemical fields. Dr. Peeters deals first with electrophoresis on strips and then with the less-familiar paper curtain electrophoresis. Factors underlying separations are examined in great detail and illustrated with many diagrams and tables. Much of the information is the author's original work which has been presented at the annual colloquia on proteins at Bruges. Dr. Marsh's article is timely in view of the increasing difficulties of obtaining personnel to cope with growing demands in clinical chemical laboratories. Simple as well as complex apparatus and processes are described and the article will be of special interest to those in charge of clinical chemical work. S. P. Bessman surveys the history, metabolism, measurement and pathological alteration of blood ammonia, and his article should stimulate interest in a subject the significance of which is not as widely appreciated as it ought to be. "Iodopathic Hypercalcæmia of Infancy" is a well-written review by J. O. Forfar and S. L. Thompsett, in which the clinical picture, biochemistry, ætiology and treatment of the disease, is covered. However, this article would possibly be more appropriate in a medical book rather than in this volume. E. J. Bigwood and associates provide a most valuable survey of amino-acid excretion in health and disease. Much of the data is quantitative and obtained by means of ion-exchange chromatography. Finally, "Bile Pigments in Jaundice" by Barbara H. Billing is an excellent account, including identification, chromatographic behaviour, methods of measurement and metabolism of these substances. Altogether, this well-produced second volume is full of interest for clinical chemists.

R. Consden

Traité Général de la Fabrication des Colles des Glutinants et Matières d'Apprêts

Par Dr. Maurice de Keghel. Nouvelle édition, augmentée et remise à jour. Pp. ii+764. (Paris: Gauthier-Villars, 1959.) 7,000 francs.

THIS new edition does not appear to be any appreciable improvement on the original version. The ground to be covered by a single author—animal glue, vegetable adhesives, casein, synthetic resins, silicate adhesives, rubber, etc., in some sections including both manufacture and use—is so wide that full references to the sources of information are essential. No references are given throughout the book, the only indication of origin being a manufacturer's name for plant, and, very rarely, an author's name, without detailed reference, for some experimental method. What basic scientific information is included is, in the main, unrelated to modern work. As an example, the conversion of collagen to gelatin is given as:

$$C_{102}H_{149}O_{38}N_{31} + H_2O = C_{102}H_{151}O_{39}N_{31}$$

This type of approach was already being superseded forty years ago when the equation was given in the text-books of that time. To-day it is not merely wrong, but also neglects the whole development of the concepts of the chemistry of macromolecules of the past thirty years.

The descriptions of plant and of industrial processes also neglect many recent developments which have changed the position of whole sections of the adhesives industry. The very large number of recipes for adhesives of various kinds are presented uncritically, with no attempts to explain the significance or function of the components used.

Certain methods of test are included. These appear to be selected at random, no reference being made to the versions accepted by the national standardizing bodies.

Neither for the manufacturer nor for the user of adhesives can this volume be said to serve a useful purpose, and less-informed readers would be misled by its many omissions and out-of-date information.

A. G. Ward

The Hollow Universe

By Prof. Charles de Koninck. (The Whidden Lectures, Series IV.) Pp. xii+127. (London: Oxford University Press, 1960.) 12*s*. 6*d*. net.

THE author of these lectures, now so attractively issued in book form, was not hampered by any rigid terms of reference, but could discuss what he liked and how he liked. Such opportunities are rare, and reflect credit upon the founder and the establishment of his trust. The theme is not altogether new; it displays the anxieties which are common enough at the ways things are going in the intellectual world. In brief, if we are nothing more than "a mere collection of occurrences", there will be (at the best) little left to wonder at in the whole domain of natural philosophy and human thought. Maybe this is so, but it makes something like nonsense of life as we known it. Prof. de Koninck is a very sensible person, and, moreover, fully alive to the claims of the positivists. His point is that in mathematical physics and everything related thereto one can, and must, be utterly impersonal, and regard Nature as purposeless.

But this does not necessarily apply to the biological sciences in which it is argued (very forcibly, in the reviewer's opinion) that an organism—that is, something provided with 'tools'—is meaningless if devoid of any teleological characteristics whatever. Incidentally, it is maintained that concentration on the lowest forms of life is not the best way to build up an understanding of Nature, any more than it is epistemologically rewarding to seek for goals and ends in plants, however fascinating, one may add, are the problems of morphology.

Thus, we are left with the tremendous question of the fundamentals of biophysics, and the advance of computers. Nobody is likely to ascribe wholly axiological judgments to mechanical systems for some time to come. That these judgments remain seem to make it probable that the universe is not entirely hollow.

F. I. G. Rawlins

Theories of Figures of Celestial Bodies

By Wenceslas S. Jardetsky. Pp. xi+186. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd. 1958.) 45*s*.

ONE of the deepest mysteries of the universe is that everything in it rotates. This remark, by the late Sir Arthur Eddington, probably explains why the theory of figures of celestial bodies has

exercised the minds of many famous mathematicians during the past century. The reader of the book under review may be surprised to find names such as Dirichlet, Liouville, Riemann, Poincaré, Volterra, and more recently, Kähler, Liapounov, Lichtenstein and Wavre associated with the subject, but this list alone indicates that the problems involved are fundamental and of the highest status. Liouville published three papers on the theory during the years 1845–1855 and Dirichlet and Riemann each published a paper in 1861. Since about this time a great deal of research has been carried out in this field and the book by Prof. Jardetsky gives a careful and faithful account of the developments that have occurred.

There are two parts, one dealing with figures of equilibrium of a rotating fluid mass, and one with other figures. The former is classical and describes the most exact methods available for determining the figures. It includes chapters on the methods of Poincaré, Liapounov, Lichtenstein and Wavre. The second part covers zonal rotation, varying figures, systems composed of fluid and rigid parts, fluid mass and centres of attraction, and figures of compressible masses. This part takes into account more physical features than the first and is to that extent more realistic, but its results are not so rigorously developed and many of the problems still await solution.

One really valuable feature of the book is that it covers material which, for the most part, was formerly tucked away in obscure or old journals not generally available. The author was the last pupil of Liapounov and thus is well qualified to bring into the light the work of his teacher in this field. He has himself contributed greatly to the subject, and there are references to his work throughout the book. Anyone working in this field will want to consult the bibliography, which is unusually comprehensive.

L. S. Goddard

The Jaws and Teeth of Primates

Photographs and Commentaries. By W. Warwick James. Pp. xii+328. (London: Pitman Medical Publishing Co., Ltd., 1960.) 105*s*. net.

THIS book is essentially an atlas of more than two hundred photographic illustrations of the skulls and teeth of representative genera of the modern Primates from tree-shrews to man, each series of illustrations being accompanied by a brief summary of the main features of the dentition. As such, it will provide a convenient work of reference for those who have not ready access to original material in museum collections. The photographs are excellent and have been most skilfully reproduced. The author's notes on the general characters, habits and habitat of each genus are derivative, but unfortunately too often based on early statements in the literature which have been superseded in more recent studies. A number of his observations, also, are surprising and even misleading—for example (in reference to the teeth of 'civilized man'), "Their beauty is regarded as their chief attribute"; in the Prosimii "the cerebral hemispheres are only slightly convoluted"; "Very few fossil Primates have been found in North or South America"; gibbons "live in large colonies which may contain fifty to a hundred members"; "Man in his present state, both mentally and physically, is very different from his ancestor a few thousand years ago"; and so forth. The appendixes on tooth development and the articular eminence raise some interesting points, but are too brief to permit any conclusions of value. Although the text contains statements open to criticism, I would repeat my main assessment, that, considered simply as a photographic atlas, the book certainly deserves commendation as a useful work of reference.

W. Le Gros Clark

Africa's Place in the Emergence of Civilisation

By Prof. Raymond A. Dart. (The Van Riebeeck Lectures.) Pp. 96. (Johannesburg: South African Broadcasting Corporation.) 2*s*. 6*d*.

THE name of Prof. R. A. Dart, who until recently was professor of anatomy at the Witwatersrand University, will always be associated with the discovery of the Taungs skull. At first some of his ideas were scouted as being fantastic, but of recent years new discoveries have brought to light other ape-men of a very early age and many of his notions have been vindicated. A short while ago he was asked to inaugurate the Van Riebeeck Lectures of the South African Broadcasting Corporation, and "Africa's Place in the Emergence of Civilisation" is the result. Prof. Dart is tremendously impressed with the "Miraculous Community-building effects of fishing upon humanity", and it is fair to say that this theme is central to the book. But naturally the first lecture deals with earlier times and the cultural differences between an ape and a first real man. Here perhaps a saying of Schweitzer to me may be of interest; Schweitzer has kept young gorillas and other anthropoid apes in West Africa and he stated that the difference between a gorilla and a man is minimal compared with the difference between a gorilla and any other anthropoid ape. Chapter 2 is concerned with the appearance of true civilization in the Near East; and then we come to fishing, which, of course, involved boats and boat building. But while this helped forward the march of progress, true civilization is based, surely, on community life and law. May I interpolate here the suggestion that civilization starts in the community life of the village but does not come to real fruition until the development of the towns, and that in Africa south of the Sahara there seem to have grown up no real towns before the coming of Europeans; this may be the fact that has caused Africa to lag behind China, northern India, the Near East and western Europe in the evolution of true 'civilizations'. Prof. Dart does not intend his little work to be the last word on an immense subject. But, just as he himself is stimulating to talk to, so his book is stimulating to read even if, for the moment, everyone may not agree with all he says.

M. C. Burkitt

Laboratory Testing in Soil Engineering

By T. N. W. Akroyd. (Geotechnical Monograph No. 1.) Pp. xx+233+32 plates. (London: Soil Mechanics, Ltd.). 50*s*. net.

MR. AKROYD'S book has been available long enough for quotations from technical reviews to appear on the cover of this edition. All, quite rightly, welcome it as a comprehensive survey, plentifully illustrated by photographs, line drawings and copies of work-sheets. It will be useful to consulting engineers who need a complete account of the kinds of test available, the problems for which the tests are helpful, and the snags that may arise. It could be useful in soils laboratories—teaching or research—where there is some interest in soil as an agricultural raw material.

H. L. Penman

CHROMOSOMES AND THE THEORY OF HEREDITY *

By Prof. C. D. DARLINGTON, F.R.S.
Botany School, Oxford

(1) Morgan's Discovery

THE crisis of the struggle in the scientific world over the chromosome theory of heredity was reached in the 1920's when Morgan's views became the subject of dispute. Morgan, with his collaborators at Columbia University, had carried out breeding experiments with the fly *Drosophila*. He claimed that by these experiments he could show that heredity, as long suspected, was indeed carried entirely by the chromosomes. It followed that these minute bodies in the cell nucleus were to be held responsible for the whole character of every living thing, plant or animal, man or microbe; and the course of evolution from the beginning had been determined by changes in these chromosomes. It was a complete scheme of determinism on Omar Khayyam lines.

This theory aroused misgiving and contradiction in many countries, especially among older men who might know the fly but certainly did not know its chromosomes. Before we look at their arguments, let us see what Morgan, and Mendel before him, had done.

Mendel had found that if he crossed two races of peas differing in two respects, as it might be $AB \times ab$, the hybrid gave germ cells of four kinds in equal numbers: *AB*, *Ab*, *aB* and *ab*. Free assortment, random recombination, independent segregation were the explanations given for this behaviour. Assortment of what? Assortment of certain 'elements' carried in all cells and passed to the germ cells. Morgan, however, beginning in 1910, found that in the fly often less than half the germ cells were of the new types, *Ab* and *aB*. The proportion was characteristic of the particular pair of elements. Assortment was not free. The elements were linked; and, if the hybrid fly happened to be a male, the linkage was complete: the elements were held together in one block.

At this point Morgan recalled that the previous year, the cytologist Janssens at Louvain had made a general proposal about chromosome behaviour. He had noticed that after the pairing of maternal and paternal chromosomes in germ cell formation they fell apart but remained touching or attached at various points which he called "chiasmata". At these points, Janssens suggested, the mating chromosomes might exchange parts by breakage and reunion. Such behaviour would give a statistical association or linkage of elements in the same chromosome of a kind already to be suspected from Bateson's breeding experiments. Conversely it would lead to the formation of new chromosomes by a recombination of parts.

Taking Janssens's hint, Morgan and his collaborators proceeded to make crosses between pairs of flies differing in many pairs of respects. They found that hundreds of elements could on this assumption be fitted into the four observed pairs of chromosomes of *Drosophila*. Moreover, if the proportion of re-grouping or crossing-over was itself assumed to be related to the distance apart of the elements along each chromosome, they found that the whole assembly fitted into fixed linear orders. Thus the elements of heredity, or using Johannsen's word the genes, could be put on a map which, like other maps, showed one how to find one's way about.

It soon seemed reasonable to advance from these direct inferences to more general principles. Morgan argued that all heredity in all organisms was carried by chromosomes as it was in *Drosophila*. Further, all chromosomes were composed of units of crossing-over or mutation which might be known as genes. Hence, he appeared to argue, the genes would add up to give the whole of heredity; and the differences between them would add up to give the whole of variation and of evolution.

(2) The Opposition

Morgan's "Theory of the Gene" appeared in 1926; its reception in England could scarcely have been more unfavourable. Seven men might have been willing to assert their belief in the chromosome theory and give their reasons for it. But against this view there were seven hundred who held a contrary opinion. The supporters felt liberated by the new theory: its opponents felt confined and oppressed by it. The grounds they gave for their opposition were both general and specific.

The general objections were that the theory was naïve and mechanical and yet self-contradictory. For it was both statistical and deterministic. It left too much room to chance yet no room to free will. In evolution, moreover, its hard particulate basis shut out the hope of any soft Lamarckian adaptation. The Lamarckian principle, we must remember, was at that time generally maintained by naturalists and physiologists and assumed by medical and social scientists. Even philosophers had their opinions, and they were on the Lamarckian side. Yet there was no room in this picture for the Lamarckian emblems, the giraffe, the salamander, or the midwife toad.

The more specific objections to the chromosome theory were also very various. The assumption that the chromosomes were alone responsible for heredity left a gap in our theory of development. Is not heredity merely a repetition of development? Yet this theory of heredity almost ignored development, and it was based on a single organism—a fly with a most disorderly development of its own. The chromosome theory also left a gap where the cytoplasm should be—where indeed European workers had found evidence of determination. Loeb, with his

* Substance of a Royal Society Tercentenary Lecture delivered on July 20.

unfortunate idea that the cytoplasm carried the solid basis of heredity while the nucleus bore only a few frills, provided a line of defence for weaker opponents of the chromosome theory.

Again it was pointed out, quite rightly, that the gene mutations of *Drosophila* could not be representative of natural variation for they were in their effects both disadvantageous and discontinuous. In the first respect they contradicted the helpful mutations of the evening primrose, *Oenothera*. In the second respect they failed to explain the universal property of continuous variation. As for the chromosomes themselves, did they not at the end of every cell division dissolve and disappear into that bag of fluid, the nucleus? As for crossing-over, the foundation of Morgan's interpretation, it was supposed to happen only in one sex and not in the other. But who had ever seen it happen anywhere? Crossing-over, like the genes themselves, was a stroke of fancy, a mathematical artefact invented to salvage a broken hypothesis.

With regard to these chromosomes, it was true, there were a variety of accounts of what they did. The two cell divisions known as meiosis, when the germ cells were formed, were especially disputable. Some believed that there were general rules; others that there were many kinds of meiosis in different groups of plants and animals. Most believed that, if there was a rule, it was that the corresponding chromosomes from the two parents paired as threads side-by-side. But a few stoutly maintained that chromosomes in the nucleus were in an endless chain which split up crosswise into segments to give $2x$ single chromosomes at mitosis, x double chromosomes at meiosis, and, by a freak of Nature, $4x$ chromosomes at mitosis making a new tetraploid. Clearly this view was of no help to those who believed that the chromosomes made heredity and were differentiated in linear structure; but it was a help to those who did not think anything of the kind. Such differences of opinion—which were described in textbooks, taught in universities and expounded in theses—created a verbal and literary jungle which had to be cleared before any general theory could be discussed.

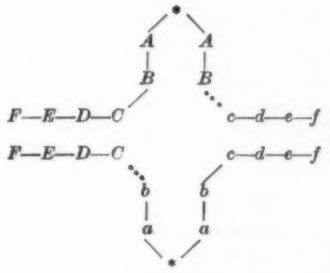
Finally, there was one specific objection which no one seemed to make. It was that genes were inferred to exist from breeding experiments with 'characters'; or more precisely, from studying the inheritance of differences of character when different parents were crossed. Yet the whole of heredity, of the genotype, was supposed to be made up of genes added together. There was a concealed gap between the analytical or differential gene and the integral genotype. In looking at the chromosomes one could see that they added up to make the nucleus. But their differences, their variations, were visibly of many kinds and degrees. The gap was revealed. How could it be bridged? Not, as it seemed to me, by pretending that it did not exist. It was necessary to work out a system of understanding life in terms of chromosomes, a system independent of experimental breeding, a system which would stand on its own feet. In this view I was strengthened by one man's opinion. "Cytology", Karl Belar said to me in 1928, "should not be the *ancilla* of genetics". That was just what I thought.

(3) Crossing-over and the Chiasma

Belar had shown that mitosis and the chromosomes themselves had a universal character, a character which must underlie the uniformity of development of plants and animals and protista. Here was a great and necessary step forward. In genetics, as in geology a century earlier, uniformity was bound up with evolution. Cytologists and geneticists too, so far as they took the chromosomes seriously, therefore liked to think that the same such universal character was true also of meiosis and underlay some uniformity in heredity. But *Drosophila* itself, with crossing-over at meiosis in the female but not in the male, faced us with the gravest objection to this view. It was possible to evade the issue for the time being. It was possible to begin with the simplest material offering experimental tests by purely chromosome criteria. For this purpose polyploid plants with large chromosomes, tulips and hyacinths, were admirably fitted. They revealed several unexpected principles. The important ones, in the present connexion, concern the chiasma.

At a certain stage in the beginning or 'prophase' of meisosis, like chromosomes come together as threads side-by-side in pairs. The association is limited to likes: it is chemically specific; and it was, I found, limited to pairs even when there are three or four of a kind. At a later stage the paired chromosomes reproduce forming double threads and at the same time fall apart. But when they do so they stick at certain points, as Janssens had said, the chiasmata.

Why do they fall apart, in one sense, and stick together, in another? Their structure gave the answer. Contrary to Janssens's view, the chiasmata always had the same structure: they were exchanges of partner between half-chromosome threads, chromatids as we call them. Further, these exchanges could be shown, on internal, cellular, microscopic, evidence to be invariably connected with a previous crossing-over between chromatids of the partner chromosomes. On the simplest assumption, therefore, chiasmata were determined by such crossing-over. The arrangement of four chromatids could be shown in a diagram that was at once genetic and cytological in its implications. With capital and small letters in sequence for the pairing chromosomes, an asterisk for the mechanical centre, and dots for the points of breakage, the diagram would be as follows:



Thus from two chromosomes, *ABCDEF* and *abcdef*, two new chromatids, *ABcdef* and *abCDEF*, had been formed, and the four chromatids would pass as chromosomes into the four germ cells formed by meiosis. The existence of these four cells would, as Janssens had put it, be justified by each of them being a unique combination of available genes different from the rest.

One could not of course prove that this principle was universally true (in those days most biologists

believed that propositions ought to be 'proved'); one could merely hope to render it increasingly probable. This hope was gradually realized. The critical configurations of several chromosomes united by, or interlocked with, successive chiasmata, the comparisons of frequencies and distributions of chiasmata and crossing-over in different organisms, in polyploids, in hybrids, with inversions and interchanges of segments of chromosome, in plants with defects of chromosome pairing and of sexual reproduction, these all helped to carry conviction. Even in *Oenothera* the occurrence of chiasmata, the inference of crossing-over, could be used to explain the modes of inheritance and the origins and kinds of mutations.

The solution of the problem of crossing-over was, however, obstructed by another, to me, unexpected but inseparable development. The study I had made of meiosis, and my interpretation of what a hundred others had seen in a great variety of plants and animals, made it clear that the chiasma had consequences which were as important as its causes. People had supposed that the chromosomes were paired at the first metaphase of meiosis because they were, in a mysterious sense, attracted to one another. This was, they thought, the climax of the sexual process. What could be more natural? But I had found that, after pairing as single threads, the chromosomes fall apart as double threads. They do not attract, they repel one another after they become double; and they are held together only by chiasmata, by the exchanges of partner between their chromatids, after crossing-over. When pairs of chromosomes fail to form chiasmata, whatever the cause, the consequence is that they lie on the spindle, unconnected and unorientated. They then fail to pass to opposite poles. Without chiasmata meiosis itself, with Mendelian segregation, the reduction of chromosome number and the alternation of haploid and diploid in the sexual cycle, all these fail to ensue.

Thus there was, in my view, a universal causal sequence: crossing-over ⟶ chiasmata ⟶ chromosome pairing ⟶ segregation and reduction ⟶ sexual reproduction.

This reversal of the mechanical interpretation of meiosis made it possible to describe meiosis in the same physico-chemical terms as mitosis. But what mattered first were its genetic implications. The new principle seemed to be true of all plants and animals (except male *Drosophila*). It therefore meant that meiosis and crossing-over had come in together, at one step. Crossing-over from its origin must have been co-extensive with sexual reproduction. What Morgan had hoped to imply I was now forced to assert. The reason why the chromosomes were divisible into units or genes was that everywhere their division into such units was a condition of meiosis and hence of sexual reproduction. This, of course, made sense in terms of selection, adaptation, and the evolution of sexual reproduction itself. For if the chromosome were not divisible into genes, if it were not capable of crossing-over, it would be inherited as a block and no genes could be revealed either to the geneticist by his experiments or to Nature by her selection. The original system would never have survived.

(4) Evolution and the Cell

It will be seen here that an evolutionary point of view was beginning to force itself into my argument.

There were several reasons why this should have happened to the student of chromosomes, by no stretch of the imagination but by hard necessity. While the experimental breeder could sort out linkage in one species, the chromosomes could reveal chiasmata in a hundred species and in every group of plants and animals. While the experimental breeder himself decided how his plants or animals should breed, the chromosome man had to pick up his cells and discover how Nature had bred them, and why, and with what effect. These were two reasons. But a third was even larger. It was that through the chromosomes there is continuity between successive generations. To the naturalist and to the experimental breeder the organism is an independent discontinuous entity. To the cytologist it is part of a continuous process. Cell division is always a step between the past and future: it is always adapted to meet conditions which do not yet exist, to produce progeny which are irrelevant to their parent's success.

Oenothera first brought this home to me. In its evolution there had been interchanges between different chromosomes, each of which succeeded by virtue of its selective advantage over its predecessors. But success depended on whether plants were inbred or outbred. Thus the hereditary mechanism and the sexual mechanism, the means of distributing and recombining differences and the means of bringing them together, must be bound up together in one system, a genetic system. In a genetic system crossing-over of chromosomes is no good without crossing of germ cells, without outbreeding. The two processes must be adjusted to one another. They must also be adjusted to the needs, not of the individual, but of the breeding group and, more particularly, of its posterity.

A second example was in the male *Drosophila*, with its suppression of crossing-over. In these flies the male, I found, had contrived an anomalous kind of meiosis without crossing-over. The chromosomes paired and separated without needing to form chiasmata. The breeding and the chromosome observations thus agreed. But how had an otherwise universal rule come to break down—and break down in the very species of organism in which the rule was first brought to light?

The reason is obvious as soon as it is pointed out. In the vertebrates or flowering plants the genes in the chromosomes are recombined once in every sexual generation. This may be once in ten months or ten years. But in the short-lived flies it happens once in ten days. That is why from the whole animal kingdom Morgan chose to work with them. But to recombine genes so often does not give any combination a chance of being properly tested. Far better to let the genes recombine in one sex and pass unrecombined through the other sex, down the other line. It is an admirable solution: new things can be made, but good things can be kept.

In the evolution of the flies it was not therefore surprising that a new type of meiosis had been developed in one sex—it had to be the heterozygous sex—in which crossing-over was suppressed. The genetic system would benefit from this modification. The original type of meiosis, on the other hand, was the common ancestral type still found in all species of plants, animals and protista, a type of nuclear division which had arisen at the origin of sexual reproduction, a type with a uniform physico-chemical character.

At the time this speculative conclusion seemed to be rash. To-day, however, we can clearly go further.

The brilliant work that is now being used to reveal the genetic structure of fungi, bacteria, bacteriophages, and other viruses makes it indeed necessary to go further. We have to say now that crossing-over of gene sequences, or nucleotide sequences, is the original property of all systems capable of evolution: and we may add that sexual reproduction, as we ordinarily understand it, is the structure built around crossing-over which has made the higher organisms possible.

This view turned genetics upside down. In the short term one could still see fertilization as the focus of life's processes. For it is at this moment in the higher plants and animals that the individual is created. But in the long term the focus was shifted to the act of crossing-over and the origin of the chiasma. For this is the moment when, we may say, the gene is created. On this event all the processes of evolution converge and from it they all diverge.

(5) Determination and Uncertainty

At an early stage in the discussion of crossing-over, the opponents of the chromosome theory objected that there was no visual or direct evidence that chromosomes did or could cross over at meiosis. When this evidence was provided they objected that there was no reason why it should happen. Fortunately the mechanical reasons were by this time only too evident. Chromosomes which pair as threads always coil around one another. Just as pairs of textile fibres spun under torsion release a part of their torsion by coiling around one another, so do the chromosomes. The part of their torsion released is in equilibrium with the rest which is stored; it is available to break the chromatids and to untwist them to a position where their broken ends can recombine in new combinations. The specificity in pairing of genes and of parts of chromosomes and the observed release of torsion at the chiasma provide for the time, place, and action, of the event inferred in *Drosophila* and of the result seen in the chiasma.

Experiments with nucleic acid starvation later indicated that the nucleic acid component of the chromosomes was the means of developing their torsion. I therefore assumed it to have the structure not of a straight column but of a spiral staircase, an assumption which has been vindicated with beautiful precision by Watson and Crick. How the molecular spiral works in detail, however, is a question we must ask later, when we have a more elaborate molecular model of the paired chromosomes.

What must be discussed now is the fact that this breakage, this crossing-over, can occur at hundreds or thousands of different places along the chromosome: indeed by one definition between any pair of genes in the whole sequence. But in a particular pair in a particular cell it occurs at only a few points, from one to a dozen; and there are conditions, even in *Drosophila* itself, where it seems to be almost fixed.

This situation, in our experience of the statistics of causal relations, seemed to be unique and significant. Its mere mechanics was easily understood. The frequency and distribution of crossing-over is characteristic of the organism. It can be regulated by the organism, by its heredity. If the chromosomes that are going to pair are regularly placed side by side in the nucleus, which sometimes happens, the amount of twisting they develop is regularly distributed and hence the crossing-over. If the chromosomes are irregularly placed, as they usually are, the crossing-over will be irregular and uncertain, as it usually is.

Thus the irregularity of crossing-over, which gives the characteristic variety of progeny in sexually reproducing organisms, is something controlled. Like the weather it shows uncertainty. But, like the weather also, we can predict it so far as we can expect to predict it. Its failure, as well as its normal conditions, show that it is a determined uncertainty. Indeed in asexual reproduction all uncertainty can be removed, and frequently is removed. Its general survival throughout the plant and animal world therefore shows that the uncertainty of crossing-over is original, is organized, and is of adaptive value. Through it indeed meiosis acts as a means of generating uncertainty.

To put the matter in another way: it is a paradox that the gene which is an organ of determinacy in life exists by virtue of a process of apparent indeterminacy. But when we examine it we find that the indeterminacy is spurious. It has been put there (if I may diverge from the present argument) by natural selection and for natural selection. It has been put there as a necessary complement of the uncertainty of the gene's mutation: together they produce adaptive variation.

The discovery of how crossing-over happens had long-range consequences beyond the previous limits of genetics. For it enabled us to split up the processes of life into two parts. First there are those concerned with determining the character of individuals. These are processes of physiology in which the greatest certainty of determination, the strictest predictability in reaction with the environment, a chemical determinism, is achieved; and secondly there are the processes of meiosis. These, reinforced by the chances of fertilization which are derived from them, determine the differences in character of individuals. They are the processes of classical genetics in which the greatest uncertainty of determination is organized and achieved.

This contrast, as I believe, between two kinds of process—the one deterministic, the other spuriously non-deterministic—also provides one of the several ways of splitting genetics into two. It means that instead of speaking of the laws of heredity, as the early Mendelians were fond of doing (making biology echo the physics of the time), we should speak rather of the 'paradoxes' of genetics. For heredity is a relation between parent and offspring which is variably compounded of the certain and the uncertain elements, according to how like the parents or grandparents may have been; indeed according to the effects of their system of breeding.

(6) The Continuing Issue

I have given an eye-witness account of a battle. I believe it is a battle that we won. The pursuit of the enemy has, to be sure, taken us far away from the original site of the conflict; and it could have taken us much further with the elasticity of the gene, the organization of the chromosome or the physiology of the nucleus. But the site of the struggle matters less than its purpose. The enemy, although defeated and dispersed, has not been destroyed. They will, in my opinion, have to be fought many times again. For mankind, if it happens to take note of the argument, will not willingly admit that its destiny can be revealed by the breeding of flies or the counting of chiasmata.

STREAMING IN CYTOPLASM DISSOCIATED FROM THE GIANT AMŒBA, *CHAOS CHAOS*

By Prof. R. D. ALLEN, J. W. COOLEDGE and P. J. HALL
Department of Biology, Princeton University, Princeton, New Jersey, U.S.A.

THE driving mechanism of amœboid movement (and its accompanying cytoplasmic streaming) has always been considered to be a property of the amœboid cell as a whole. We wish to report new findings which demonstrate that the cytoplasm can be removed from an amœba in a state in which it displays continuous and vigorous streaming *in vitro* for periods as long as an hour. Our experiments vividly demonstrate the inadequacy of the sol-gel or ectoplasmic contraction theory of amœboid movement[1,2], and lead to the conclusion that the cytoplasm of amœboid cells is structurally organized into subcellular 'units of streaming', the nature of which will be discussed here.

The phenomenon of persistent, continuous streaming in the cytoplasm of ruptured amœbæ (*Chaos chaos* and *Amœba proteus*) was described briefly by the senior author in 1955 (ref. 3). Since that time there has been no hypothesis to explain this phenomenon, and a more complete account of it was postponed until a satisfactory hypothesis could be tested. The recent development of the 'fountain zone contraction theory' of amœboid movement has offered for the first time a satisfactory explanation for several perplexing aspects of amœboid movement[4,5]. According to this theory, the endoplasm begins to contract actively (that is, develop tension) as it enters the 'fountain zone' just posterior to the hyaline cap, where it becomes everted to form the ectoplasmic tube. The tension developed between the anchored advancing rim of the ectoplasmic tube and the movable endoplasm displaces the latter anteriorly. Continuous streaming is maintained by propagation of the contraction posteriorly along the endoplasm at the same velocity as that at which the endoplasm advances relative to the pseudopodial tip. At the anterior end of the cell the contraction phase of the contractility cycle expresses water (and solutes) to form the hyaline cap. The hyaline cap fluid must then be returned to the recruitment zone (tail endoplasm), where it is believed that the extension phase of the contractility cycle occurs[5]. The mobility of the plasmalemma with respect to the ectoplasmic tube[3,6] demonstrates the existence of a fluid channel (the hyaline ectoplasm) which could serve to transport the hyaline cap fluid to the posterior portion of the cell. The endoplasm has been shown to possess structure sufficient to transmit tension[7,8]. The details of evidence on which the fountain zone contraction theory is based is being presented elsewhere[5].

With the fountain zone contraction theory in mind, we have re-examined the phenomenon of streaming in cytoplasm dissociated from the amœba and have obtained quantitative data which satisfy many of the predictions of the fountain zone contraction theory.

Cytoplasm is freed from the cell by rupturing the cell membrane while the amœba itself is held in a quartz capillary to prevent the cytoplasm from becoming dispersed. The most successful method is to draw an intact amœba carefully into the fine bore of the capillary and then to shatter the capillary with a sharp scalpel under a pool of paraffin oil. The oil seals the capillary at both ends, thereby trapping the cytoplasm in a closed container isolated from outside forces. The capillary is drawn in two stages under an oxygen flame: first, from 6 mm. inside diameter to 1–2 mm., and then in a smaller flame to 75–200μ.

The experiment is easy to perform; students in the cellular physiology course at Princeton usually succeed after a few attempts in obtaining dissociated cytoplasm preparations which stream continuously

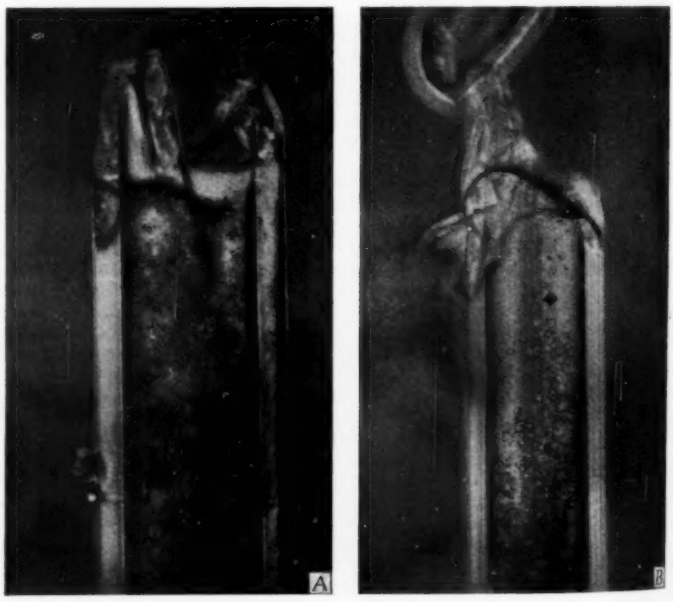


Fig. 1. Photographs of actively streaming cytoplasm dissociated from the giant amœba, *Chaos chaos*, contained in a broken capillary submersed in a pool of paraffin oil. (*A*) a preparation in which the capillary was shattered at the ends of the amœba to form a protoplasm–water interface. The marker is 50μ long. (*B*) similar to *A*, except that the capillary was shattered peripheral to the ends of the amœba. The arrow shows one of the loops of cytoplasm which stream out into the water and return to the main cytoplasmic mass (see text). The marker is 10μ long

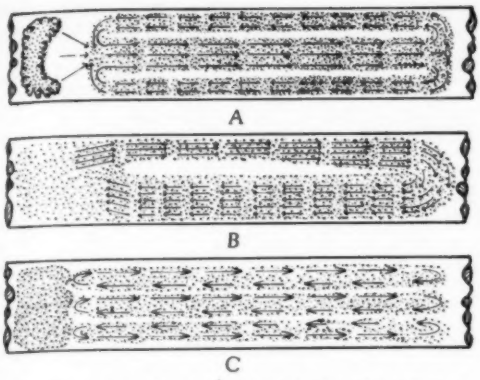


Fig. 2. Diagrams to show : (*A*) the fountain streaming pattern of stage I ; note the remnants of the plasmalemma on the left side of the diagram ; (*B*) stage II, showing the loop-streaming pattern ; (*C*) stage III, after division along the longitudinal axis of some of the loops has occurred to increase the number of streaming units

for a half-hour or more after rupture of the cell. The only difficult part of the procedure is to draw the amœba into a sufficiently narrow capillary without rupturing its membrane prematurely. It is also essential that the cell fill the bore of the capillary, in order to prevent excessive dilution of the cytoplasm with water or other aqueous medium. It seems to make little difference to the outcome of the experiment whether the capillary is shattered directly over the two ends of the amœba (as in Fig. 1*A*) or slightly peripheral to the amœba (Fig. 1*B*), as the shock of breaking the capillary usually is sufficient to rupture the plasmalemma. The dissociated cytoplasm seems to stream equally well whether in contact at the ends with oil or water.

While it is possible to perform this experiment with any large, free-living amœba, *Chaos chaos* cytoplasm displays the most vigorous movement and 'survives' for the longest periods, ranging from 10 min. to an hour. Our best results have been obtained from *C. chaos* which were raised in Pringsheim solution[6], fasted 24 hr., washed in ion-free water, and cooled to 2° C. for about 12 hr. prior to an experiment. Amœbæ treated in this manner begin to stream within minutes after return to room temperature, and for a half-hour thereafter tend to remain monopodial. In this condition they are more readily drawn into capillaries without premature rupture. None of these treatments is absolutely necessary, and, in fact, the experiment will work with amœbæ taken directly from wild cultures. The low-temperature pretreatment renders the cells less fragile during transfer to capillaries, and the ion-free water reduces the tendency of the cytoplasm to become vacuolated following rupture of the plasmalemma.

Whether or not the cell exhibits streaming prior to rupture of the plasmalemma, the shattering of the capillary results in vigorous streaming which may reach a velocity double that in the intact cell.

The pattern of streaming usually goes through at least two of three recognizable stages during its 'survival' within the capillary. In the first stage, the cytoplasm circulates in the 'fountain pattern' typical of that seen in intact cells which are unable to become attached to a solid substratum. During this stage, any remnants of the plasmalemma are transported by the outer or 'ectoplasmic' stream to the former posterior end of the cell, where they can often be seen as a folded structure (Fig. 2*A*).

Within the first few minutes, the second stage of streaming begins by the appearance of an even number of 'units of streaming' which pass back and forth in a more or less orderly manner. Detailed observation of these 'units' shows that they are, in effect, bands or loops of cytoplasm, the bends of which are located usually at the former anterior region of the cell (Fig. 2*B*). It is difficult to determine how the change from the fountain pattern to the loop pattern occurs, but it seems likely that the cytoplasm breaks up into what correspond roughly to radial sectors. The units of streaming are definitely not channels through which 'plasmasol' flows, for solid cylinders of streaming cytoplasm are coherent and do not disperse in moderate amounts of water (see Fig. 1). They exhibit 'plug-flow' velocity profiles remarkably similar to those shown by the endoplasmic stream of the intact cell[7]. Sometimes thin cytoplasmic strands, recognizable only by moving rows of inclusions, come out into the water at the ends of a preparation similar to Fig. 1*B*, and follow a U-shaped course through the water and back into the main mass of dissociated cytoplasm.

During the third stage (Fig. 2*C*), there is a marked retardation of streaming velocity and a tendency (not seen in all preparations) of the units of streaming to divide longitudinally or 'tear'. This 'tearing' of the cytoplasmic structure appears to be a result of the development of unequal tension from contraction in the cytoplasm. The tension is relieved by tearing along a longitudinal fault in each streaming unit, resulting in its division into two units which thereafter move independently. The thinnest streaming units produced by such divisions might be called 'fibrils', although they are visible only as a linear aggregate of particles which move in single file.

If it is assumed that the U-shaped bands of cytoplasm arise from radial division of the fountain streaming pattern, it can then be shown that these units of streaming behave as predicted by the fountain zone contraction theory. In order to relate the behaviour of the units of streaming to the events in the intact cell, we shall refer to the cytoplasm approaching the bend of a loop as the 'endoplasmic arm' of the loop and the cytoplasm receding from the bend as the 'ectoplasmic arm'. This designation appears justified from the velocity profiles across the adjacent ecto- and endo-plasmic arms of a typical unit of streaming shown in Fig. 3. The velocity profile across the endoplasmic arm is remarkably similar in shape to that across the endoplasmic stream of the intact cell, as measured by Allen and Roslansky[7] ; that across the ectoplasmic arm is flatter, as expected of a more rigid gel. The different velocity profiles almost certainly indicate a difference of consistency between the less rigid endoplasmic arm and the more rigid ectoplasmic arm of the loop, even though the profiles cannot be interpreted strictly in terms of classical flow theory (cf. ref. 7).

The fountain zone contraction theory predicts that the endoplasmic arm should shorten and thicken in passing through the bend. That endoplasm does shorten is shown by the fact that it is displaced toward the bend at 2–3 times the velocity at which the ectoplasmic arm recedes from the bend. (Velocity ratios of greater than 1 and less than 2 were recorded on film, but the positions at which these ratios were recorded were too far from the bend to be certain that the bend had remained stationary. No case has

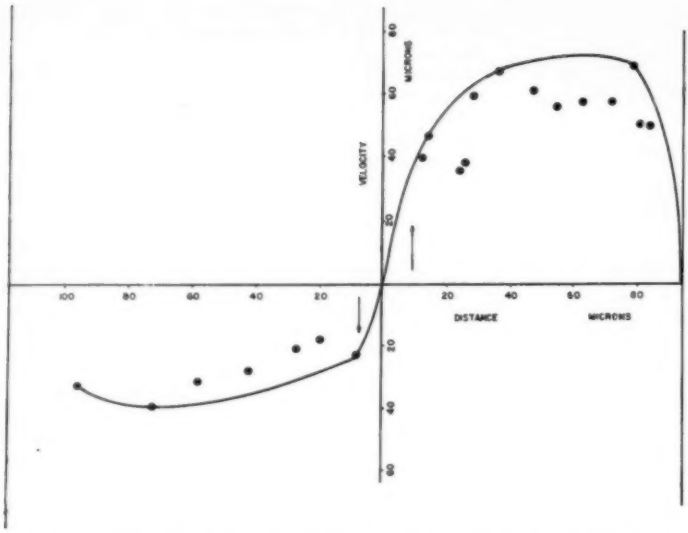


Fig. 3. A velocity profile across the endoplasmic (right) and ectoplasmic (left) arms of a broad, U-shaped loop of cytoplasm, measured approximately 150μ posterior to the bend. Note that the maximum velocity of the endoplasmic arm (streaming toward the bend) was approximately double that of the ectoplasmic arm (streaming away from the bend). The sum of the velocities was about twice the velocity of endoplasmic streaming of intact cells

been found of more rapid streaming away from the bend than toward it. The same velocity relationships hold true in the intact cell (see Fig. 4).) The shortening must occur where the velocity decreases; Fig. 4 shows that this decrease of velocity occurs at the junction between the endoplasmic arm and the bend. In favourable cases it has also been possible to measure a pronounced thickening of the cytoplasm in such a unit of streaming as it passes through the bend. In the example represented by Fig. 3, the cross-sectional area of the ectoplasmic arm was 3·8 times that of the endoplasmic arm, based on measurements of diameter. These changes in length and cross-sectional area are similar to those which have been shown to occur in the intact cell[5].

Besides the geometry changes mentioned above, there are more direct indications that a tension-producing contraction occurs at the bend of these units of streaming. Of particular significance is the fact that when streaming of cytoplasmic loops occurs in spurts, activity can be detected first at the bend of the loop, where a conspicuous shortening occurs. Immediately afterward, the cytoplasm moves toward and away from the bend. Thus the temporal sequence of events indicates that the shortening process at the bend is responsible for pulling the endoplasmic arm toward, and pushing the ectoplasmic arm away from, the bend.

In order to result in streaming, the contraction must remain localized in the vicinity of the bend. This can be accomplished only if the contraction is propagated posteriorly along the endoplasm of the intact cell (or endoplasmic arm of a streaming unit) at the same velocity as that at which the endoplasmic stream advances relative to the bend. Ordinarily, the contraction is inconspicuous because it remains at the bend. Occasionally, however, the contraction is seen to be propagated along straight sections of cytoplasm from one end of the capillary to the other. Occasionally also, the bend itself moves with the contraction, especially if the ectoplasmic arm is held in place. When this occurs, the bend is 'whipped' along the capillary by the propagated contraction.

A further manifestation of contractile activity is a 'twitching' movement which occurs just prior to cessation of streaming. Localized contractions may also give rise to very complicated rotatory and other movements.

There is evidence of a syneresis associated with the contraction at the fountain zone (stage I), or at the bends of cytoplasmic loops (stage II), which is probably analogous to hyaline cap formation in the intact cell. In the presence of traces of calcium ions, an increasing number of clear vacuoles forms after the membrane of the cell is ruptured. The vacuoles form as tiny tear-shaped bodies which suddenly 'blow up' like balloons in the fountain zone (stage I) or at the bend of a streaming unit (stage II). If the formation of these clear vacuoles is the result of synæresis, then the argument that hyaline cap formation results from synæresis associated from the fountain zone contraction is strengthened. It is then reasonable to suppose that this synæresis occurs also when insufficient calcium is present to cause the formation of a visible vacuolar membrane around the fluid expressed from the interstices of the contracting cytoplasm.

The streaming of amœba cytoplasm in the absence of the plasmalemma and ectoplasmic tube as structures completely eliminates the sol-gel or ectoplasmic contraction theory as a possible explanation for

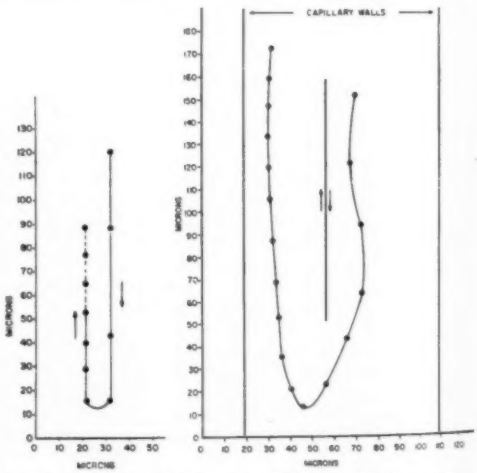


Fig. 4. Left: the positions occupied at intervals of 1 sec. by an inclusion as it moved forward in the endoplasm and backward in the ectoplasm relative to the tip of a pseudopod in an intact *Chaos chaos*. Right: the same for an inclusion carried in a U-shaped loop of dissociated cytoplasm. Note in both cases the retardation at the junction between the endoplasmic arm (streaming toward the bend) and the bend itself

streaming in cytoplasm dissociated from amœboid cells. This theory demands the presence of an intact ectoplasmic tube to convert ectoplasmic shortening into a pressure gradient.

The results of the present work provide additional evidence that the endoplasm of the amœba is not a structureless sol, but rather a material which is structurally and physiologically organized and capable of carrying on cytoplasmic streaming at the sub-cellular level. The partial disintegration of cytoplasm dissociated from giant amœbæ into motile 'units of streaming' provides evidence which is not only compatible with, but also provides further verification for, the fountain zone contraction theory of amœboid movement[5]. Each unit of streaming consists of a U-shaped loop or band of cytoplasm with a bend at which a contraction occurs. The two arms of the loop show a difference of consistency ; the contracted or ectoplasmic arm is more rigid. Therefore, when the bend is held stationary and the contraction is propagated in one direction, the cytoplasm of the loop streams through the bend in the opposite direction.

We believe that the principle upon which the movement of amœba cytoplasm has been shown to depend may be widely applicable to many other cases of cytoplasmic streaming, and that the demonstration of this principle at the sub-cellular level represents an important step toward an understanding of the mechanism of amœboid movement and cytoplasmic streaming.

This investigation was supported by a Research Grant, C-3022, from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

[1] Pantin, C. F. A., *J. Mar. Biol. Assoc. U.K.*, **13**, 24 (1923).
[2] Mast, S. O., *J. Morph. Physiol.*, **41**, 347 (1926).
[3] Allen, R. D., *Biol. Bull.*, **109**, 339 (1955).
[4] Allen, R. D., "Amœboid Movement", in Vol. 2 of "The Cell", edit. by Brachet, J., and Mirsky, A. E. (Academic Press, in the press).
[5] Allen, R. D., *Exp. Cell Research* (in the press).
[6] Griffin, J. L., and Allen, R. D., *Exp. Cell Research* (in the press).
[7] Allen, R. D., and Roslansky, J. D., *J. Biophys. Biochem. Cytol.*, **6**, 437 (1959).
[8] Allen, R. D., *J. Biophys. Biochem. Cytol.* (in the press).
[9] Andresen, N., *C.R. Trav. Lab. Carlsberg, Sér. Chim.*, **29**, 435 (1956).

THE NATIONAL ENGINEERING LABORATORY, 1960

OPEN DAYS

THE third open days of the National Engineering Laboratory (formerly the Mechanical Engineering Research Laboratory), East Kilbride, Glasgow, held on June 15 and 16 and the evening of June 17, were attended by more than 2,200 representatives of industry, universities, technical colleges and other research organizations. All the work at East Kilbride and Thorntonhall was on display, and about a hundred items were specially featured. There was also a display describing the facilities available for carrying out sponsored investigations for individual firms.

The behaviour of metals under stress is the concern of the Materials Division, where the aim is to provide the designer with basic data on the strength of materials, particularly under fatigue and creep. In many components and structures, fatigue cracks can form very quickly, and it is necessary to know the conditions under which these will grow if the component is to be designed efficiently with economic use of material. Investigations, using 10-in. wide sheets under tensile fatigue loading, have shown that low stresses ($5 \pm 1\frac{1}{2}$ tons/in.^{2} for mild steel) are sufficient to cause cracks to grow, and this growth may be continuous or intermittent. When growth is continuous, for overall crack-lengths up to one-eighth of the total width of the sheet, the rate of growth is dependent on the cube of the alternating stress and on the current crack-length.

Fundamental studies of the mechanism of growth of fatigue cracks in mild steel indicate that the growing crack is made up of many small independent cracks which have no relation to the initial cracks found in brittle fracture or to the inclusion cavities of ductile fracture. It is thought that elementary fatigue cracks form as a result of the development of a sub-grain structure, the sub-grains being an essential feature of cyclic dislocation movement.

Under stress at high temperatures, materials undergo a slight continuous elongation, known as creep. To provide data for the adequate design of components which will operate under these conditions, as, for example, in steam-power plant, the Laboratory is examining the creep characteristics of typical engineering metallic alloys. Their properties, under simple and generalized loading conditions, under relaxation conditions where the total strain is kept constant, and under conditions leading to actual fracture, are being studied and the relation between creep-rate, stress and time has been established for several materials.

The development of techniques and apparatus for the accurate measurement of large water-flows in pipes is necessary for the efficient design and operation of hydraulic machinery. One technique employs propeller current meters, mounted in a predetermined pattern over the cross-section and each rotating at a speed proportional to the local velocity of flow. In conventional propeller meters, bearing friction at low flow-rates makes the meter calibration non-linear and dependent on time. The Fluids Division at the Laboratory has developed a frictionless meter incorporating a hydrostatic pressurized bearing which provides a dynamic film of water between rotating and fixed parts. Static friction is eliminated and linear calibration is now obtained over a wide range of flow-rates. It is hoped that precision moulding of the impeller will eliminate the need for calibration of individual meters when the first one or two impellers have been calibrated.

A robust dual-element thermometer has been devised to measure temperature between 0 and 50° C. to within a few millidegrees centigrade under industrial conditions. It is used in conjunction with an a.c. bridge having transformer ratio arms, and the circuit can be arranged to eliminate the effect of long supply leads and bridge outer leads. The temperature is obtained from the ratio of the temperature coefficients of resistance of the copper and minalpha

elements, and the need for a highly stable external resistor (and consequent temperature-controlled enclosure) is eliminated.

Heat transfer is an essential process in the operation of a wide range of mechanical engineering equipment, and it is part of the concern of the Heat Division to obtain more accurate data for use in the design of such equipment. A particular example of fundamental importance in this field is the flow of heat between a pipe and a fluid passing through it. Studies of the effect of abrupt changes of section and right-angled bends in pipes have shown that for low flow-rates, and particularly for laminar flow, there is an increase in the heat-transfer coefficient after the discontinuity (in one instance the coefficient rose to ten times its normal value). This increase may extend up to 50 pipe diameters downstream of the discontinuity. For higher flow-rates, the increase in the heat-transfer coefficient is comparatively small and does not extend far along the pipe.

To enable heat-exchange apparatus to be designed more efficiently, there is need for a more accurate knowledge of the properties of the gases to be used, and the Division is producing tables of thermodynamic and transport properties of technically useful gases. The National Engineering Laboratory is the United Kingdom representative in the preparation of an internationally acceptable set of steam tables; a new skeleton table, extending up to 1,000 atmospheres and 800° C., is being drawn up. To reconcile the different experimental data and to keep them consistent with the thermodynamic relations, an equation of state, valid for the vapour state, has been devised, based on *P-V-T* data; this equation represents pressure as a double-power series in density and temperature, and all the other properties can be derived from it.

One of the aims of the Metrology Division is to improve the accuracy and performance of machine tools and mechanisms such as gears, lead screws, ball-bearings and universal joints; as part of the Division's work, new types of precision measuring instruments, using optical, mechanical, electronic and pneumatic techniques, are developed. Particular attention is being given to the application of moiré fringes to precision measurement, and automatic control of machine tools (moiré fringes are produced when light is shone through two similar gratings placed face to face with their lines not quite parallel). Using a photo-electric pick-up, linear or rotary movement can readily be converted into a corresponding electrical signal, and this system can be used as a basis for comparing the relative motion of two elements in a mechanism—the signal representing the error in relative motion can then be used to actuate a correction mechanism. A system of this type is now providing continuous and automatic correction of the errors in rotation of the worktable of a commercial gear-hobbing machine. A photographic process is used to produce the lines on the precision radial gratings for this and other applications; previously the lines were ruled on an aluminium-coated glass plate, using a circular dividing machine. A small segment of the grating is first ruled and is then mounted concentrically with a photographic plate secured to the table of the dividing machine. By alternate exposure of the plate to a beam of light passing through the master segment, and indexing of the table through an integral number of grating pitch spaces, a complete photographic copy is obtained. This method eliminates short-term periodic errors in the grating, and the time required for production can be reduced considerably (for a radial grating with 10,800 lines, from 4 days to about 3½ hr.).

Pressurized air bearings are being employed to reduce friction in instruments and machine tools. They can be used to provide a high-precision axis of rotation in journal bearings, or to enable a carriage to travel accurately in a straight line along a slideway. A typical application of the second type has been the development of a flat air bearing to support the ¼-ton worktable of a horizontal milling machine. Cutting performance of the machine is unimpaired, while it has been possible to reduce the size of the traverse motor from 2 to 1/50 h.p.

A clear understanding of the processes of lubrication and wear is essential if machines are to run efficiently and have a long service life. The Lubrication and Wear Division is studying one of the major forms of wear in machinery—the abrasive action of hard fine particles. This may occur, for example, in internal combustion engines, if the air is not properly filtered at intake, and in agricultural machinery, under the action of soil particles. Work on the resistance to abrasion of a wide range of carbon steels and cast irons has shown that in general an increase in hardness of the material improves the resistance, but in some cases other factors, such as graphite or eutectic cell-size in cast irons, may be equally or even more important. Other work has shown a correlation between resistance to abrasive wear and to fretting corrosion (the wear occurring between two contacting surfaces subject to repeated relative oscillatory motion), and it may ultimately become possible to predict fretting corrosion resistance from abrasive wear tests.

Under unsteady loads, such as are encountered in internal combustion engines and in high-speed turbomachinery, the position of the shaft in a journal bearing varies in the bush, and excessive vibration and bearing failure may result. Inductive transducers are being used to detect and trace the shaft movement under the action of various loads, including a combined gravity load and unbalanced rotating load. The rupture of the lubricant film in the unloaded part and the consequent change in the hydrodynamic conditions in the bearing appear to be responsible for the observed variations in shaft movement.

The work of the Plasticity Division is directed towards obtaining a better understanding of the behaviour of metals during plastic deformation. This will lead to more effective utilization of metals, and in metal-working processes to improved techniques and more efficient machine tools.

In forming operations the temperature distribution within the metal must be known and controlled to avoid excessive rises of temperature which may cause undesirable changes in the properties of the product —for example, 'hot shortness' in extruded material. A combined theoretical and experimental technique is being used to determine the rates of rise of temperature in a material during extrusion, and a programme has been prepared for the Laboratory's *Deuce* computer to obtain the temperature distribution as a function of time under various extrusion conditions.

Investigations have shown that under high hydrostatic pressure, materials behave in a more ductile manner than at atmospheric pressure, and that some normally brittle materials, for example, bismuth and magnesium, can be successfully cold-extruded inside

a chamber containing fluid at high pressure; a superimposed hydrostatic pressure of 2–3 tons/in.^{2} may be sufficient to prevent cracking of the extruded product for low reductions in area. This technique may have several commercial applications, particularly in the working of new and difficult materials.

Apparatus to generate pressures up to 100,000 atmospheres in conjunction with temperatures up to 3,000° C. has also been built. This will be used to investigate the forming of new materials of technical and scientific interest under simultaneous high pressure and high temperature. Typical effects of such conditions are the transitions of graphite to diamond and hexagonal boron nitride to borazon. In the case of borazon, its great hardness and resistance to oxidation at high temperature give it considerable industrial potential. R. I. LITHGOW

NEWS and VIEWS

Psychology at Leicester : Prof. S. G. McK. Lee

DR. S. G. MCK. LEE, who has been elected to the new chair of psychology in the University of Leicester, is a South African by birth and gained his early education and teaching experience in psychology at the University College of Natal. His war service, during 1940–46, included four years as navigator with the South African Air Force and the Royal Air Force. Dr. Lee has a close knowledge of the language and culture of the Zulus, among whom he carried out studies in social psychology and cultural influences on personality. His reports on the 'crying hysteria' of Zulu women and the cultural significance given to their dreaming are well known. He has standardized intelligence tests and a version of the thematic apperception test for Africans. After taking his Ph.D. at Birkbeck College, London, where he also held a teaching post, Dr. Lee became in 1955 senior lecturer in psychology at the University College of Fort Hare, where, however, he reluctantly decided that political restrictions on African education would soon make his work nugatory. He returned to London, to join the staff of Bedford College, where he has taught for the past four years. In his recent research work he has brought together his training as an experimental psychologist and his experience in clinical psychology by investigating experimentally, in collaboration with Dr. D. W. Forrest, the effects of repressed interest and emotion on perception. He is now (in association with Dr. G. M. Carstairs, of the Medical Research Council) applying one of the experimental techniques developed for that purpose to a study of psychological aspects of hypertension.

C.S.I.R.O. Division of Physical Chemistry : Dr. S. D. Hamann

DR. S. D. HAMANN has been appointed chief of the Australian Commonwealth Scientific and Industrial Research Organization's Division of Physical Chemistry at Fishermen's Bend, Victoria. He succeeds Dr. K. L. Sutherland, who has resigned to become director of research for the Colonial Sugar Refining Co., Ltd. Dr. Hamann was born in Christchurch, New Zealand, in 1921. He graduated M.Sc. with honours at Canterbury College, having specialized in chemistry and physics. He spent more than four years in the Royal New Zealand Navy, being engaged almost continuously for three years in operational and technical research on radar. At the conclusion of the War, Dr. Hamann studied at the University of Manchester, and was awarded a Ph.D. degree in 1950. Having returned to Australia, he immediately joined the Commonwealth Scientific and Industrial Research Organization's High-Pressure Laboratory in the Chemical Engineering Department of the University of Sydney. He has been in charge of this Laboratory since 1952. Dr. Hamann has represented Australia at meetings of the International Union of Pure and Applied Chemistry in Zurich (1955) and Munich (1959).

C.S.I.R.O. Division of Forest Products : Mr. S. A. Clarke

MR. STANLEY A. CLARKE, chief of the Division of Forest Products of the Australian Commonwealth Scientific and Industrial Research Organization, retired from active duty on August 26. Born in Perth, Western Australia, in 1900, an engineering graduate of that State and an associate member of the Australian Institute of Engineers, Mr. Clarke has been associated with the Division of Forest Products since 1929. In that year he was seconded to the newly formed Division of Forest Products as officer-in-charge of seasoning and utilization. The following year he transferred to the permanent staff of the Division, and in 1931 was appointed as deputy chief of the Division. He held this position until 1944, when he succeeded the late Mr. I. H. Boas as chief of Division. Mr. Clarke has won the esteem and confidence of the Australian timber industry, and has established a reputation both in Australia and overseas for his ability to apply his engineering and scientific outlook to problems in the utilization of forest products. Devoting himself in the first instance to the problems of saw-milling, timber seasoning and timber grading, he afterwards became an authority on such widely diverse activities as those of the pulp and paper, fibre board and particle board industries. Since 1930 he has participated actively in the work of the Timber Industry Committee (formerly Timber Sectional Committee) of the Australian Standards Association, being appointed as chairman to the Committee in 1944. His advice has been widely sought by such countries as New Zealand, India and the United Kingdom. Mr. Clarke has taken a keen interest in the United Nations Food and Agriculture Organization's work in relation to forest products, and in addition to being chairman of the Asia–Pacific Regional Committee of Forest Products Research he is a permanent member of the Technical Panel on Wood Technology of the Organization.

Reflexion of Signals from *Echo* I

A TEAM of scientists from the Post Office and the Royal Radar Establishment, Malvern, succeeded on the evening of August 29 in receiving signals at Malvern reflected from the Earth satellite *Echo* 1 (see *Nature*, August 27, p. 741). The signals, which included both unmodulated carrier waves and speech

transmissions, were transmitted from the Bell Telephone Laboratories at New Jersey, U.S.A. Prof. A. C. B. Lovell, of the Jodrell Bank Radio Astronomy Station, announced that signals from New Jersey had been received about a week ago, using the 250-ft. radio telescope. The present tests at Malvern are of particular interest since the radio aerial used was only 20 ft. in diameter.

The National Chemical Laboratory

The report of the National Chemical Laboratory, 1959 (London: H.M. Stationery Office, 1960. 4*s*. 6*d*. net), begins with a report of a steering committee set up in October 1958, which made several recommendations. Thus the Committee proposed the transference of the collection of industrial bacteria to the Torry Research Station and the disbanding of the research section. The greater proportion of the National Chemical Laboratory's effort is to be devoted to work on the thermodynamic properties of pure materials. Work on high polymers is reduced to the investigation of certain aspects. Work on selective membranes is to be continued. There will be closer co-operation with the National Physical Laboratory. The work on corrosion, always a valuable part of the research in the National Chemical Laboratory, is continuing. A Radio-Isotope Applications Group works at Harwell. The report as a whole contains some interesting material, usually presented in sufficient detail to be intelligible to non-specialists.

Science Horizons

Commencing with the August issue, the title of the monthly review of scientific progress in the United States of America, *Atoms for Peace Digest*, issued by the United States Information Service, American Embassy, London, W.1, has been changed to *Science Horizons*, to reflect the broadened editorial content of the journal. For some time now the journal has been reporting developments in space research, electronics and technology generally, in addition to specific developments in nucleonics, and these are now to become regular features. Topics discussed in the August issue include ram-jet engines for supersonic airliners; a world-wide, all-weather navigation system, based on Earth satellites; and the 'laser', a light amplifier. *Science Horizons* is to be published during the third week of every month, but will be dated a month ahead, that is, for example, the October issue will be distributed during the third week of September.

International Scientific Radio Union

The thirteenth General Assembly of the International Scientific Radio Union (U.R.S.I.) is being held at University College, London, during September 5–15. The Union is in the nature of a federation of national scientific bodies interested in the radio aspects of scientific research and development. The present assembly is being held at the invitation of the Royal Society which, through its British National Committee for Scientific Radio, is responsible for making the arrangements for this meeting, under the chairmanship of Mr. J. A. Radcliffe. The previous General Assembly was held at Boulder, Colorado, in 1957 (see *Nature*, **181**, 465; 1958), and Dr. L. V. Berkner, head of Associated Universities, Inc., New York, was elected president of the Union for the current term, and is presiding over the plenary meetings in London. The scientific programme of the forthcoming assembly is organized under seven commissions, the titles and chairmen of which are: I, Radio Measurements and Standards (Dr. B. Decaux); II, Radio and Troposphere (Dr. R. L. Smith-Rose); III, Ionospheric Radio (Dr. D. F. Martyn); IV, Radio Noise of Terrestrial Origin (Prof. R. A. Helliwell); V, Radio Astronomy (Prof. A. C. B. Lovell); VI, Radio Waves and Circuits (Prof. S. Silver); and VII, Radioelectronics (Prof. W. G. Shepherd).

The British Pteridological Society

During the latter half of the last century, searching for and growing wild varieties of British ferns were wide-spread pastimes. In 1891 a group of Lakeland hunters formed themselves into the Northern British Pteridological Society and attracted members from all over Britain. During those early years, the emphasis was on varieties and their cultivation, and this is still regarded as an important and integral part of the activities. In recent years, the scientific side has gradually developed and increased in importance. This movement has been greatly accelerated during the past year, which has seen big changes and re-organization in the Society. The importance of operating in a much wider field has been realized and the Society's aims now include the study of world ferns and fern allies. The recording and dissemination of information on the British Pteridophyta still remain, of course, a major activity. In addition, an attempt is being made to build up a herbarium of varieties in existence to-day. An annual excursion for a week to parts of the country where ferns abound has long been a popular institution in the Society. Additional meetings are held during the year to cater for members whose interests are taxonomic rather than horticultural. Day field meetings and indoor meetings are held in the London area, and a separate northern meetings secretary and committee organize field meetings for members in the north of England and in Scotland. The current annual subscription is 10*s*. Members receive the *British Fern Gazette*, published annually, and, occasionally, other publications. The secretary is Mr. J. W. Dyce, 46 Sedley Rise, Loughton, Essex.

Mushroom Growing

The Ministry of Agriculture, Fisheries and Food is to be congratulated on the new edition of its bulletin on "Mushroom Growing" (Bull. No. 34, seventh ed., price 6*s*. H.M.S.O., London, 1960). The sixth edition was issued some six years ago, and, as noted in the foreword, both before then and since, the mushroom-growing industry has seen many important changes, specializations and refinements in its practices. Indeed, "Changes in practice are occurring so rapidly that no account can be reasonably up to date for very long". It is in the light of the many recent and contemporary changes and innovations that the present guide has been written under the chairmanship of Mr. F. W. Toovey, director of the Glasshouse Crops Research Institute. The essential facts and procedures are well and fully set out, and are illustrated by some excellent photographs.

Botanical Garden in Tashkent

A new botanical garden was founded in 1950 in Tashkent's northern suburb. Of the total projected area of 80 hectares, 40 hectares are now occupied by a dendro park containing 1,300 species of trees and shrubs belonging to the local, European, Far Eastern, Chinese and North American flora. The dendro park

contains several artificial ponds and it is traversed by 12 km. of roads. As described by F. N. Rusanov (*Priroda*, **7**, 61; 1959) the dendro park contains many well-acclimatized, rare and valuable plants and hybrids. The park is at the service of the whole of Central Asia.

Submarine Oil Drilling

ACCORDING to M. K. Kalinko (*Priroda*, **11**, 25; 1959), the first attempt to extract petroleum from rocks underlying the bottom of the Caspian Sea were made in the region near Baku in the beginning of the nineteenth century. The workings, protected by two wooden frames lowered at the bottom of the sea, were destroyed by a storm in 1825. In 1910, in an attempt to infill a shallow bay, petroleum was discovered, which led to drilling in the region; but planned survey and undersea petroleum extraction began only in 1935. At the present time, undersea extraction is carried out on a large scale in eight separate areas in the region of Baku, and in 1958 this work extended to the eastern part of the Caspian Sea. Outside Russia, the submarine extraction of petroleum began in Japan in 1888, but it reached its maximum development in the Gulf of Mexico, California, Venezuela, and the Persian Gulf. Special surveying and prospecting methods are now employed for the investigation of the possible offshore petroleum provinces or 'aquatories'. The author of this article gives a general survey of the world's offshore petroliferous provinces, which in his opinion may yield more petroleum than the overland provinces.

Use of Gamma-Radiation Sources for the Sterilization of Pharmaceutical Products

A REPORT issued by the Association of the British Pharmaceutical Industry (B.M.A. House, Tavistock Square, London, W.C.1. Pp. 17) describes the findings of a working party set up by a number of pharmaceutical firms and other interested bodies, including the Atomic Energy Research Establishment, to investigate whether pharmaceutical products and containers could be usefully sterilized on a commercial scale by using gamma-radiation. The results of the tests are summarized in a convenient form in this report. A dose of $2{\cdot}5 \times 10^6$ rads (from cobalt-60) appeared to be adequate to achieve sterility, but it caused changes in many of the substances tested, and in many cases rendered them unsuitable for use. It is suggested that the method may be usefully applied in particular cases, but the results, in general, are not considered sufficiently encouraging to justify continuation of the present investigation on a group basis.

Molecular and Crystal Structure Models

A BIBLIOGRAPHY on molecular and crystal structure models used extensively in research and as teaching aids has recently been published (U.S. Department of Commerce: National Bureau of Standards. Monograph No. 14: Bibliography on Molecular and Crystal Structure Models. By Deane K. Smith. Pp. iv+7. Washington, D.C.: Government Printing Office, 1960. 15 cents). The references are grouped into four broad classifications—models in general, static models, dynamic models, and constructional devices—and the static model classification is, in addition, subdivided into closed molecular (Stuart type), closed packing, open molecular, open crystal structure, open with parallel rods, polyhedral, and miscellaneous models. The list of references is preceded by brief explanations of the various types of model which indicate the more significant articles relating to them.

Hæmatology of the Central Australian Aborigine

VARIOUS hæmatological observations on central Australian aborigines have been made by J. R. Casey-Smith, of the Department of Anatomy, University of Adelaide (*Austral. J. Exp. Biol. and Med. Sci.*). One group contained some thirty nomadic males living under primitive conditions for most of the year. The second group consisted of about twenty males and fifteen females living under urban conditions, on an almost European diet. The nomadic group's diet had a high iron content. The hæmoglobin levels of a control group of Europeans in Central Australia were also measured. Hæmoglobin and hæmatocrit values of the nomads were higher than those of the 'urban' males; neither sex of the 'urban' community differed significantly from the European controls. The erythrocyte counts showed no significant difference between the two male groups. Hæmoglobin-levels of sixteen Europeans living at Alice Springs were almost the same as those of the 'urban' males. The mean corpuscular volumes and the mean corpuscular hæmoglobins of the nomads were just greater than those of the Europeans, but the 'urban' natives did not differ significantly from either the nomads or Europeans. The mean corpuscular hæmoglobin concentrations did not differ significantly between the groups or from European standards. Erythrocyte osmotic fragilities showed an increase in hæmolysis at $0{\cdot}5$ N, but otherwise were not significantly different from those of Europeans. The aborigines did not differ significantly among themselves. The erythrocyte sedimentation-rates were greater in all groups of aborigines when compared with Europeans, but the nomads' values were less than those of the urban groups.

Virus-Streak Disease of Tomatoes

THE syndrome of the streak disease of tomatoes in Ontario has been shown to be double-virus in nature, resulting from the synergistic action of tobacco mosaic virus and potato virus X (MacNeill, B. H., and Ismen, Hikmet, *Canad. J. Bot.*, **38**, 9; 1960). Expression of the disease was found to depend on both virus components of the complex, a variation in strain characteristics of either tobacco mosaic virus or potato virus X being reflected in the intensity of their interaction in the tomato. The extreme severity of streak in the greenhouse and field tomato crops in Ontario is associated with what appears to be a geographical strain of tobacco mosaic virus. This virus is characterized by the induction of primary local lesion in petunia without subsequent systemic invasion of that host. Air temperature profoundly affects the development of streak, symptoms being masked above the critical temperature of 26° C. This phenomenon is a true masking effect with both viruses becoming latent in the tomato host.

Excavation of La Grotte de Rochereil

IT is a pleasure to direct attention to a straightforward cave excavation where the finds are of prime importance and are not dependent on a statistical or other theory which has to be demonstrated ("La Grotte de Rochereil", by P. E. Jude, *Mém.* 30, *Arch. Inst. Paléont. Humaine*. Masson et Cie., Paris).

The site is not far from Périgueux, and the cultures present are the late Magdalenian (mostly *M6*) and Azilian. The fauna indicates a very cold period during the Magdalenian occupation, succeeded by an unpleasant period of transition leading to a more temperate climate. The implements, both in flint and bone, are numerous and characteristic, and many of the bone tools are decorated. Human remains occur in each culture-level. A distribution map of Azilian finds in south-west France is given, and it would seem to rule out the theory that there was direct connexion between the Azilian of south-west France and the early Mesolithic cultures (Obanian, etc.) of Ireland and western Scotland, linked by a series of cultural sites now under the sea. Actually, Azilian sites are conspicuously absent from the coastal areas of south-west France. "La Grotte de Rochereil" is a worthy volume to add to the other *Mémoires* of the Institute of Human Palæontology; it is well illustrated.

Research on Tinplate in Australia

The Broken Hill Proprietary Company, the sole producer of tinplate in Australia, has made a grant of £6,000 per annum to support tinplate research in the Commonwealth Scientific and Industrial Research Organization. The grant will be used to support investigations at the Organization's Division of Food Preservation and Transport at Homebush, New South Wales; this is the main centre in Australia for research on tinplate packaging problems. One project to be undertaken will be a study of the reasons why certain foods sometimes cause intense corrosion or deep pitting of tinplate. Until 1957, when the Broken Hill Proprietary Company opened its new plant at Port Kembla, Australian supplies of tinplate were all imported; now, however, the Company supplies hot-dipped tinplate for the majority of the food cans manufactured in Australia, and it will be possible for research workers to trace the history of the plate right back to the steel ingot; this will be very useful in corrosion studies.

Physiological Role of Fast Transfer Reactions

To inquire into the possible physiological role of fast transfer reactions in eliciting specific interactions in ordered macromolecular structures, a series of seminar lectures was held at the Massachusetts Institute of Technology during the spring term of 1960. Included were lectures on electron and energy transfer mechanisms and on current concepts of proton transport mechanisms of immediate biophysical and biochemical significance. Properties of water and ice crucial for proton transfer were reviewed. Six lectures of a more general nature dealt with aspects of macromolecular interaction properties. Abstracts of these lectures and pertinent references are available free of charge. Requests should be sent to Prof. Francis O. Schmitt, Department of Biology, Massachusetts Institute of Technology, Cambridge 39, Mass.

Foundation of Plant Geography

Students of the history of botany will note with interest and appreciation the production of a facsimile edition of the essay by Alexander von Humboldt and A. Bonpland on the "Géographie des Plantes", dated Paris 1805, but believed not to have been issued until two years later. This work, the original of which is not readily accessible, has been issued by the Society for the Bibliography of Natural History, as Sherborn Fund Facsimile, No. 1 (c/o British Museum (Natural History), London, S.W.7; price 10*s*.). Humboldt is generally regarded as the founder of modern plant geography, and here, in the boldly printed pages of his "Essai", some of his original comprehensive observations and ideas are set out.

Society for Applied Bacteriology: Officers

At the annual meeting of the Society for Applied Bacteriology, the following officers were elected: *Hon. President*, Mr. G. Sykes; *Hon. Secretary*, Dr. Ella M. Barnes; *Hon. Treasurer*, Mr. G. Elis Jones; *Hon. Editors*, Dr. S. E. Jacobs and Dr. D. J. Jayne-Williams; *Hon. Publications Manager*, Mr. A. H. Walters; *Hon. Advertising Manager*, Mr. E. J. Mann; *Members of Committee*, Dr. Eve Billing, Mr. N. J. Butler, Mr. B. M. Gibbs, Dr. N. D. Harris, Dr. J. R. Norris, Dr. Jane Meiklejohn, Dr. A. M. Paton, Dr. Joan Taylor, Dr. G. C. Ware; (*ex-officio*), Dr. C. A. E. Briggs, Mr. D. A. McKenzie.

The Animal Health Trust: Educational Awards

The following Animal Health Trust educational awards for 1960–61 have been announced: *Wellcome Fellowships*: R. B. Taylor (University of Liverpool), to undertake research into tolerance and the development of immunity in animals at the Department of Zoology, Cambridge; I. J. F. Stobo (University of Edinburgh), to study problems of the early weaned calf, with particular reference to nutrition and the development of the digestive organs, at the National Institute for Research in Dairying, Shinfield, Reading. *Research Training Scholarships*: R.A.S.E. Research Training Scholarship to R. M. Connan, of the Royal Veterinary College, to study general pathology at the Department of Animal Pathology, Cambridge; A. D. Allen Memorial Scholarship to G. W. Osbaldiston, of the University of Queensland, to study animal nutrition and general husbandry at Cambridge; Dr. W. N. M. Foster, of the University of Edinburgh, to undertake research into the problem of 'quidding' in Border Leicester sheep and to complete his veterinary degree at the Royal (Dick) School of Veterinary Studies, Edinburgh; R. M. Griffin, of the Federal Department of Veterinary Research, Northern Nigeria, to continue investigations into contagious bovine pleuropneumonia and to attend the course for the diploma of bacteriology at the London School of Hygiene and Tropical Medicine; I. F. H. Purchase, of the University of Pretoria, for work in the field of animal anæsthesia at Cambridge.

University News: London

Mr. G. Belyavin, senior lecturer at University College Hospital Medical School, has been appointed to the University chair of bacteriology tenable at that School.

Mr. E. J. Ambrose, senior lecturer at the Institute of Cancer Research: Royal Cancer Hospital, has been appointed to the University readership in physical chemistry tenable at that Institute. Dr. F. A. Holton, a member of the scientific staff of the Experimental Radiopathology Research Unit of the Medical Research Council at Hammersmith Hospital, has been appointed to the University readership in veterinary biochemistry tenable at the Royal Veterinary College.

The title of reader in chemistry has been conferred on Mr. G. M. Timmis in respect of his post at the Institute of Cancer Research : Royal Cancer Hospital ; and that of reader in mechanical engineering on Dr. C. R. Webb in respect of his post at Queen Mary College.

Pittsburgh

THE following appointments have been announced : Dr. Theodore Holstein, to be professor of physics ; Dr. Robert L. Wolke, to be associate professor of chemistry ; Dr. David S. Feingold, to be assistant professor of bacteriology ; and Dr. Robert Silverman, to be assistant professor of mathematics.

Announcements

DR. MAURICE EWING, director of the Lamont Geological Observatory at Columbia University, has been awarded the John Fleming Medal of the American Institute of Geonomy and Natural Resources.

MR. F. E. SHOTTON, deputy county advisory officer, Norfolk, in the National Agricultural Advisory Service, has been appointed director of Terrington and Kirton Experimental Husbandry Farms, Terrington St. Clement, King's Lynn, Norfolk, in succession to Mr. A. C. Owers, who is resigning on November 1.

DR. W. M. HAMPTON is retiring this month from the position of managing director of Chance Brothers Ltd., Glass Works, Smethwick, Birmingham, after 43 years with the company. He joined the firm as a chemist in the then newly formed research laboratories, and has been successively manager of the Optical Glass Department, director of research, technical director, 1940, and managing director, 1953. Dr. Hampton will remain a member of the Chance Brothers board.

DR. N. E. ODELL, of Clare College, Cambridge, has been appointed by the South East Asia Treaty Organization to the chair of geology in the University of Peshawar, which is tenable for a year or more.

DR. JOHN D. KENDALL has been appointed director of European research laboratories to be established by the Minnesota Mining and Manufacturing Co., Ltd. Dr. Kendall, who is a graduate of the Imperial College of Science and Technology, London, has been responsible for many years for the direction of organic chemical research at Ilford, Ltd., and is known for his work on photographic sensitizing dyes and developers.

A SYMPOSIUM on "Flow Measurement in Closed Conduits" will be held at the National Engineering Laboratory, East Kilbride, Glasgow, during September 27–30. Attendance is by invitation.

THE first International Congress of Food Science and Technology will be held in London during September 18–21, 1962, under the presidency of Lord Rank. Further information can be obtained from Col. Francis J. Griffin, 14 Belgrave Square, London, S.W.1.

THE Nutrition Society (Scottish Group) is to hold a symposium on "Food Habits in Britain" at the Institute of Physiology, University of Glasgow, on September 24. Further information can be obtained from Dr. J. Davidson, Rowett Research Institute, Bucksburn, Aberdeen.

A POSTGRADUATE course on "Recent Advances in Steroid Chemistry" is being organized by the Kingston Technical College. There will be eleven weekly evening lectures by distinguished visitors commencing on September 28. Further information can be obtained from N. Lindop, Department of Chemistry, Biology and Geology, Kingston Technical College, Fassett Road, Kingston-upon-Thames.

PROF. W. T. ASTBURY, professor of biomolecular physics in the University of Leeds, will deliver the ninth Procter Memorial Lecture of the Society of Leather Trades Chemists at Leeds on September 23. The annual meeting of the Society is being held in the University of Leeds during September 23 and 24.

THE Institute of Physics and the Physical Society is arranging a conference on "Diamond Physics", to be held at the J. J. Thomson Physical Laboratory, University of Reading, during September 22–23. During the meeting Prof. R. V. Jones will deliver the Duddell Medal address ; he will speak on "Some Developments of the Optical Lever".

AN informal discussion on the "Ultracentrifugation of Biological Macromolecules", organized on behalf of the Colloid and Biophysics Committee of the Faraday Society, will be held at the Medical School, University of Birmingham, during September 14–15. Details can be obtained from Dr. D. R. Stanworth, Department of Experimental Pathology, Medical School, Birmingham 15.

A FOUR-DAY course for trade union officials on "The Industrial Uses of Radioisotopes" is to be held at the United Kingdom Atomic Energy Authority's Isotope School, Wantage, Berks, during September 20–23. Provision is also being made to accommodate students. Further information can be obtained from the General Secretary, Trade Union Congress, Congress House, Great Russell Street, London, W.C.1.

THE first joint symposium, under the auspices of the Internation Union of Biochemistry and the International Union of Biological Sciences, has been organized by Profs. O. Lindberg and T. W. Goodwin and will be held at the Wenner-Grens Institut, Stockholm, during September 12–17. The subject will be "Biological Structure and Function". Further information can be obtained from Prof. T. W. Goodwin, Department of Agricultural Biochemistry, University College of Wales, Aberystwyth.

A NEW approach to training senior scientists and technologists in administration, based on case studies, has been developed at the Northampton College of Advanced Technology, London. This serves as a basis of the advanced course on "Direction and Supervision of Scientific and Engineering Personnel", which will begin on October 12 and will consist of meetings at fortnightly intervals, for a whole day at a time, until June 7, 1961. The organizers and tutors are Mr. A. C. Leyton, Mr. Z. M. T. Tarkowski and Miss Avice V. Turnbull. Further information can be obtained from Mr. Z. M. T. Tarkowski, 703 Frobisher House, Dolphin Square, London, S.W.1.

ERRATUM. In the obituary notice of Prof. Max von Laue in *Nature* of August 27, p. 738, a misunderstanding led to an alteration being made in the reference to von Laue's obituary of Haber. The full quotation is : "Themistokles ist in die Geschichte eingegangen nicht als der Verbannte am Hof des Perserkönigs, sondern als der Sieger von Salamis. Haber wird in die Geschichte eingehen als der geniale Erfinder desjenigen Verfahrens, Stickstoff mit Wasserstoff zu verbinden . . ." (*Naturwiss.*, 22, 97 ; 1934).

BRITISH COLONIAL TERRITORIES

"THE Colonial Territories, 1959–1960", while still providing the convenient compendium of information to such publications as "Colonial Research", is of slighter interest to the scientist and technologist than previous issues (pp. vi+147. Cmnd. 1065. London: H.M. Stationery Office, 1960. 6*s*. 6*d*. net). The section, "Research and Surveys", has been omitted and also that dealing with international relations, and information on the latter topic is now scattered through the other chapters. Recruitment to the public service in overseas territories through the Colonial Office decreased during the year: 1,083 appointments were made as against 1,335 in 1958, and of these, 250 were teachers, 48 agricultural officers, 93 engineers and 100 doctors. Vacancies at the end of the year totalled 866, compared with 964 at the end of 1958. Training courses and attachments were arranged for 2,239 officers of overseas governments, a decrease of 196. Expenditure on development and welfare schemes and on research was about £24·4 million, and the estimated cost of approved technical assistance projects in British dependent territories in 1960 under the United Nations Expanded Programme of Technical Assistance is 1·1 million dollars compared with 1·06 million dollars in 1959. Under this programme 120 experts and 20 fellowship holders were provided, of whom 71 experts and 1 fellowship holder were provided by the World Health Organization, 23 experts and 11 fellowship holders by the Food and Agriculture Organization, 9 experts and 4 fellowship holders by the Bureau of Technical Assistance, and 6 experts and 1 fellowship holder by the United Nations Educational, Scientific and Cultural Organization. On March 31 some 30 specialists from the United States were serving in the African territories, and the United Nations Special Fund has approved grants of 700,000 dollars for a survey of a multi-purpose dam site on the Niger River, 850,000 dollars for the engineering faculty of the University College of the West Indies and 250,000 dollars for a survey of siltation conditions of Demerara River, British Guiana.

The number of students at the universities and university colleges, apart from the 330 at the University College of Sierra Leone, which was not included last year, increased by 385, and of the total 213 are at the Royal University of Malta, 1,268 at the University of Hong Kong, 695 at the University College of the West Indies, 1,112 at the University College, Ibadan, and 880 at the University College of East Africa. The Royal Technical College of East Africa admitted 288 students, and there are more than 1,000 students at the Nigerian College of Arts, Science and Technology. Under the Fulbright agreement, nine American teachers were attached to schools in British Guiana, Malta, Western Nigeria, Northern Rhodesia, Uganda, Zanzibar and the West Indies, two senior American research workers were attached to university and research institutions in Kenya and Northern Rhodesia, and one scientist continued work on the preservation of wild life in Uganda. Fulbright travel grants enabled lecturers from University College, Ibadan, and the University College of East Africa, and a graduate from Kenya to visit the United States for research and lecturing, and sixteen students from Cyprus, Malta, Jamaica, Hong Kong, Kenya, Nigeria, Tanganyika and Uganda were awarded grants for one year of study in the United States. The use of the services of the Overseas Visual Aids Centre has grown rapidly.

Under medical and health services the report records a localized outbreak of 117 cases of typhoid fever in North Borneo, but otherwise the incidence of communicable disease fell, though in an outbreak of cerebro-spinal meningitis in Northern Nigeria there were 1,450 deaths. Major schemes for the complete eradication of malaria continued, and extended trials of recent drugs for treatment of leprosy continued in Nigeria, Kenya, Uganda and elsewhere. Studies of acute anterior poliomyelitis have indicated a very high degree of natural immunity among the locally born population of the Seychelles and St. Lucia. Mass campaigns against yaws using penicillin have been strikingly successful, but despite intensive and persistent attempts to maintain safe vaccination-rates, smallpox remains endemic in Africa.

SCIENCE AND EDUCATION IN BRITAIN

TWO short but interesting debates on science and education were held in the House of Commons on July 27. The first of these, arising out of a prayer to withhold assent to University Court Ordinance No. 350 (Universities of St. Andrews, Glasgow, Aberdeen and Edinburgh) governing regulations for research students and the appointment of research Fellows, related to the part of the universities of Scotland in the revival and stimulation of industrial activity. Mr. A. Woodburn, who moved the prayer, questioned whether they are doing enough and quoted opinion that neither Scottish industry nor the Scottish universities had quite come to terms with this age of science and technology. While 17 per cent of students in Scotland took a first degree in science, for science and technology the figure was only 11 per cent, and research students in science and technology were only 8 per cent of those in Great Britain, and only 10 out of 17 Department of Scientific and Industrial Research awards to advanced course students were taken up in 1960 compared with 251 out of 344 in the United Kingdom, and grants in support of special researches at Scottish universities, while amounting to £267,000, were only 7 per cent of the total for Great Britain. This latter figure was attributed to the lack of applications and proposals, and not to any restrictions on the part of the Department. Mr. Woodburn also remarked that

only 2·7 per cent of research and development in private industry proceeds in Scotland, and that the Department of Scientific and Industrial Research is responsible for 81 per cent of research in shipbuilding and engineering. Some 58 per cent of engineering students in the University of Glasgow found work out of Scotland; for Edinburgh the figure was 69 per cent, and for Aberdeen 64 per cent.

Lady Tweedsmuir, who supported the Ordinance, asked for more information about university expenditure on research and on teaching. At Aberdeen in 1959–60 there were 91 full-time and 24 part-time postgraduate research students, compared with 77 and 22, respectively, in 1954–55. Research Fellows were very few, and the Scottish nniversities asked of this Ordinance that they should have the same facilities as English universities enjoyed to attract research workers. Mr. G. M. Thomson, who also asked for further information, questioned whether the pattern of professorships in the Scottish universities is appropriate to modern needs. He also stressed the importance of adequate finance, and suggested that more provision is required for the training of postgraduate students, better facilities for those with a technological qualification to qualify for research awards and a free flow of information between the universities, Parliament and the interested public. Sir Myer Galpern challenged some of these views, and suggested that much information is already available; he claimed that the Scottish universities already produce research students of very high calibre. The Joint Under-Secretary of State for Scotland, Mr. Niall Macpherson, intervened to point out that the Government regards the matter as one for the universities alone, and stressed its desire not to infringe their independence in matters of academic policy. Miss M. Herbison, referring to applications for grants for special researches from the Department of Scientific and Industrial Research, pointed out that of 25 in 1959–60 from Scottish universities, 13 came from the Royal College of Science and Technology, Glasgow. Dr. A. E. Thompson also supported this plea for more co-operation between the universities and the technical colleges, and for much greater facilities for exchange of staff between universities and other research institutions.

Another debate, on an adjournment, was opened by Mr. T. F. Peart, who raised specifically the responsibilities of the Minister for Science and his relation to the Ministry of Education. After stressing the importance of the contribution which British scientific man-power should make to Colonial development, Mr. Peart urged the full implementation of the De La Warr report on higher education in agriculture, stressed the need for adequate teaching of science in the teacher-training colleges and for a good supply of graduates in science from the universities who would take a teacher's diploma. He said he is concerned about the teaching of science in girls' schools and the supply of science teachers and equipment for such schools, and asked for a major debate on the place of science in our economy and the functions and responsibilities of the Minister for Science and the Minister of Education. The Parliamentary Secretary to the Minister of Education, Mr. K. Thompson, replying for the Government, welcomed the debate, but claimed that much is already being done to relieve the shortage of science teachers. He said that a very large proportion of the expansion of the teacher-training colleges would be in the numbers of teachers qualified to teach science at various levels in the schools. He agreed that we could also use part of this expanded output to provide an introduction to science in the primary as wel! as secondary modern and grammar schools. In the main, there are, he said, three levels of teacher-training for science courses in the training colleges: the ordinary curriculum; one providing for a main study of a science subject; and the advanced specialist course in a science subject. The number of teacher-training college students in mathematics and science has increased from 600 in 1956–57 to more than 1,100 in 1959–60, and the net increase in mathematics and science graduates in 1959 was 612. Plans for university expansion should provide an 80 per cent increase in science graduates in the period up to 1960.

ADULT EDUCATION

A HEARTWARMING tribute to the work of local enthusiasts for continued education has been paid by the Joint Committee for Adult Education of Queen's University of Belfast. In its twenty-eighth annual report it states that "it is commonplace, when adult education is discussed to-day, to lament the crusades of the founding fathers. It is even commoner to deprecate the decline of local responsibility and initiative, and the abdication of sustained and particular studies, in favour of the armchair and the printed or televised magazine. The experience of the Joint Committee and its officers is in contrast to all this".

These words, to the University Council for Adult Education, are in general borne out by the reports of other extra-mural departments*.

In spite of problems of finance, the total number of courses shows a slight increase, from 4,612 in 1957–58 to 4,741 in 1958–59. The volume of work has remained almost stationary for the past three years, but these figures mask the continued decline of tutorial classes, a trend which has gone on steadily for several years, as is shown by the figures:

1954–55	1955–56	1956–57	1957–58	1958–59
983	959	950	904	851

Its decline must cause disquiet, but it is encouraging that tutorial classes organized in collaboration with the Workers' Educational Association have shown a less-sharp fall (from 619 to 600) than others (from 285 to 241).

Television has been regarded as a serious rival to adult education, and blamed for the decline in tutorial classes. It is therefore interesting to find the University College of North Wales, Bangor, postulating the possibility that the coming of television may prove an advantage to adult education in so far as its better programmes tend to encourage "viewers to seek further enlightenment on the topics treated in them while the inferior programmes drive the more intelligent citizen to seek better pastures".

* Universities Council for Adult Education. Report on the Year 1958–1959. Pp. 34. (Bristol: W. E. Salt, Honorary Secretary and Treasurer, The University, 1960.)

The number of residential courses shows a 12 per cent increase over the 1957–58 figure, although, as last year's total was smaller than the 1955–56 and 1956–57 figures, the gain in part represents a recovery in lost ground. The increase is entirely in the shorter residential courses, that is, those of not more than seven days in length. At Cambridge, where Madingley Hall has been used to the full extent of its availability, the most interesting and promising developments are thought to be those in residential courses.

Table 1. Trends in Adult Education, 1953–59

	1953–54	1954–55	1955–56	1956–57	1957–58	1958–59
Religion	180	211	222	252	251	270
Biology	245	258	252	274	274	294
Language and literature	636	685	675	681	689	719
Visual arts	—	—	—	370	383	421

Table 1 confirms certain trends which have become discernible in adult education during the past few years. The reasons for these changes are not clear, but speculation indicates that the hydrogen bomb and affairs in Africa throw up moral problems that turn the minds of some men and women to a deeper study of the religious beliefs which have been a formative force in Western civilization. The Dead Sea scrolls combine the excitement of archæological discovery with the higher criticism. The contraction of the world prompts the serious and sympathetic study of religions that become less strange the more they are understood. It is possible that courses with a religious content satisfy some of the interests that previously found an outlet in the study of philosophy and of international affairs. The upward trend of courses in the visual arts has become clearly discernible.

There has been a marked improvement during the past few years in much of the accommodation in which extra-mural work takes place. Gradually, it is being recognized that adult education needs, and deserves, accommodation of its own, and the number of adult education centres steadily increases, some provided by universities, some by local education authorities, and some by voluntary organizations. The University of Leeds has established non-residential centres for extra-mural work at Bradford and Middlesbrough, each under the oversight of a member of the Department's teaching staff acting as warden. The University of Liverpool has made the Royal Institution available as a centre for extra-mural courses.

The tendency towards meeting the needs of special groups is discernible in the class programme of extra-mural departments, as well as in residential courses. The Edinburgh report contains an apposite comment on this development: "There is a considerable demand for classes of a semi-vocational nature but in this field extra-mural educational activities are limited to work for which the university is particularly suited. Classes in Agricultural Law, Income Tax, Estate Duty and Company Administration, Industrial Law, Advanced Management, Effective Reading and Programming for Digital Computers indicate a change in the pattern of extra-mural work but they should be regarded as a widening of the scope of such work rather than a change of emphasis". Oxford has embarked on a scheme, with support from the Leverhulme Trust, for the preparation of relevant and conveniently accessible teaching material—hitherto lacking—in trade union education. Special studies are being undertaken in three industries in Oxford's area—coal mining, engineering, and road passenger transport—in the hope that the resulting material will be suitable for publication for use by classes throughout Great Britain.

THE MELLON INSTITUTE

THE annual report on research, by the chairman, Mr. M. B. Ridgway, to the Board of Trustees of the Mellon Institute for the year ended February 29, besides a review of activities and of independent research during the year, of the research services and research for public welfare, includes a list of research personnel and of publications of Mellon Institute members during the year*. Fundamental research now represents about 20 per cent of the Institute's research effort, and two new fundamental research groups, one in continuum mechanics and visco-elasticity and the other in theoretical chemistry, began activity during the year. Among the continuing groups, expansion was chiefly in polymer science, metal physics and radiation, while in the applied research groups those in the science of metals also expanded. The independent research staff increased from 56 to 77, and 16 groups are now engaged in fundamental research. At the end of the year there were 42 industrially sponsored fellowships and projects in applied science with a staff of 210. Research services employed 26 professional scientists, and exclusive of part-time employees the total staff of the Institute was 536; expenditure for fundamental and applied research during the year was 5,781,000 dollars.

In studies on enzymatic hydroxylation a third hydroxylating co-factor has now been isolated from cultures of *Mycobacterium* 607 which reverses the inhibition of hydroxylation caused by tetracycline and also that caused by 2 : 4-dinitrophenol. In inorganic chemistry osmium and ruthenium complexes were characterized and investigations started on the co-ordination complexes of rhenium, iridium and platinum, while many basic compounds have been prepared in polycrystalline form by homogeneous hydrolysis in the range 170–300° C. Investigations of the structure and properties of macromolecules continued, and the stress–strain curves for a wide variety of cross-linked polymers revealed substantial agreement with the theoretically predicted form when measurements were made under conditions permitting reasonable approach to equilibrium. In metal physics an improved method was developed for evaluating the lattice spacings of gold-based and silver-based alloys, and the study of strain-induced transformation in silver–zinc alloys in the β-phase range was

* Mellon Institute Research 1959–1960: The Annual Report of the Chairman, Matthew R. Ridgway, to the Board of Trustees of the Institute for the Fiscal Year ended February 29, 1960. Pp. vi+33. (Pittsburgh: Mellon Institute, 1960.)

completed, using metallographic and X-ray procedures. In organic chemistry, triphenyl-, trimethyl- and tributyl-phosphonium-fluorenylides, as well as triphenylarsoniumfluorenylide and dimethylsulphoniumfluorenylide were prepared and their properties and reactions studied, as well as the protonation of weak bases by sulphonic acid type of ion-exchange, while in physical chemistry exciton theory was applied to the vibrational spectra of molecular crystals, and the atomic co-ordinates and anisotropic thermal vibration parameters of α-quartz at room temperatures were precisely determined with accurate X-ray intensities from an almost ideally imperfect single crystal.

Other work on the science of metals included the study of the solubility of nitrogen in the iron–manganese system, basic high-temperature strengthening mechanisms for ferrous alloys, the evolution of new ultra-high strength steels with a total alloy content not exceeding 9 per cent and the research and development of solid state devices for power utilization. Research for public welfare ranged over such fields as air purification, the protection of artists' materials from discoloration and deterioration, treatment of effluents, industrial hygiene, abatement of water pollution and examination of the toxicology of new organic chemicals. Among general research fellowships and projects may be mentioned those concerned with the development of new solid catalysts and the improvement of existing granular absorbents from bone products, the fundamental properties of porcelain-enamel glasses, computer components, special films and film-forming starch products, determination of the critical requirements for the ideal industrial felt fabric, the synthesis of new insecticides of the methylenedioxyphenyl group for use in combination with pyrethrins, the synthesis and evaluation of textile resins and softeners and the synthesis of lubricants and hydraulic fluids with improved stability to oxidation and heat. Chemical work on the genesis of petroleum continued, thermal degradation studies of the polyene pigments were initiated and work continued on the preparation and properties of novel types of silicone polymers and co-polymers, on organofunctional silicone chemistry and studies of the structure and properties of organosilicone compounds.

THE NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

IN an official Government publication of a generation ago dairy research was defined as "the study by scientific methods of the dairy industry and its products, and of the fundamental principles underlying its operations". It is now almost fifty years since the National Institute for Research in Dairying was founded at Reading, and its annual report for 1959* illustrates how thoroughly that conception of the all-embracing nature of dairy research has been developed at what has undoubtedly become one of the most famous research stations in the world.

The dairy industry begins at the farm with the management of grassland and the production of feeding-stuffs for milk production, with the breeding, management, nutrition and health of the dairy stock and with the actual production and handling of the milk itself. When the milk leaves the farm, other important sections of the dairy industry are concerned with its transport and distribution, its manufacture into dairy products and with the engineering and machinery required for these purposes; of course, the dairy industry in all its branches is concerned that milk and the products made from it should be bacteriologically sound and of the highest possible nutritive value. In all this there is immense scope for scientific research, both fundamental and applied, and at the National Institute, where there is now a total staff of about 370, of whom 114 are scientific or experimental officers: the three principal departments which deal mainly with milk production problems are the Dairy Husbandry, Feeding and Metabolism and Physiology Departments. The remaining seven principal Departments — Bacteriology, Chemical Microbiology, Chemistry, Physics, Radiobiochemistry, Nutrition and Engineering—are concerned partly with milk production and partly with the quality and handling of milk and with the nature and properties of milk products and the scientific principles underlying their manufacture. The main part of the report, occupying about 100 pages, gives an account of the work of each of these ten Departments and also of the Statistics Section and the Experimental Dairy, but for those who prefer to see at a glance what an important contribution to knowledge the Institute makes even in the relatively short period covered by this report, a brief outline is given of some of the most recent research findings. This outline occupies only three pages, but it includes some 23 separate items ranging from subjects such as haymaking with a forage harvester, rumen development in calves and work on teat-cup liners of various different designs, to the part played by certain hormones in lactation, the ultra-high-temperature sterilization of milk and problems associated with the manufacture of Cheddar cheese.

The research of a basic nature includes work in which radioactive isotopes have been used to investigate some of the enzyme mechanisms in the mammary gland mitochondria and also to obtain information on the proportions in which various lower fatty acids are formed from hemicellulose and α-cellulose in the rumen. It includes also the establishment of an infusion technique whereby solutions of lower fatty acids can be introduced into the rumen continuously for long periods and relationships worked out between the amounts and nature of the fatty acids in the rumen and the yield and composition of the milk. In research of a more applied nature, studies have been made of matters such as the circulation-cleaning of pipe-line recorder milking equipment and the development of a rapid method of determining lactose in separated milk and condensed whey which depends on infra-red absorption. Studies have been made also of the effect of mastitis on the yield and composition of milk.

* University of Reading. Report of the National Institute for Research in Dairying, 1959. Pp. 154. (Shinfield, Reading: National Institute for Research in Dairying, The University, Reading, 1960.) 4s.

Towards the end of the report a list is given of nearly 200 papers published by members of the staff during October 1958–September 1959. In the notes which deal with staff matters, reference is made to the fact that in 1959 Prof. R. G. Baskett took up his appointment as director of the Institute, and a brief but well-deserved reference is made to the retirement of Dr. A. T. R. Mattick, who has served the Institute and the dairy industry with distinction for some forty years.

J. A. B. Smith

NEW AMERICAN OBSERVATORIES

In order to make astronomical research facilities available to those many universities which cannot themselves afford the necessary expensive equipment, the National Science Foundation is providing financial support for the construction of two major observatories, the National Radio Astronomy Observatory at Green Bank, and the Kitt Peak National Observatory near Tucson, Arizona.

Green Bank, in Deer Creek Valley, West Virginia, is at an elevation of 2,600 ft., ringed by mountains rising to 4,000 ft., effectively shielding the site from man-made radio noise. The climate is mild, and there are no high winds which would make operation of a large radio telescope difficult. An 85-ft. telescope was brought into operation in October 1958, and since then construction of a 140-ft. telescope, laboratories, residences and workshops has been under way. The reflecting surface of the 85-ft. telescope, made of aluminium sheet, is accurate to $\frac{1}{4}$ in. and operates down to wave-lengths as short as 3·5 cm., at which the telescope beam width is 7 min. of arc. Both telescopes are on equatorial mountings. The moving mass of the 140-ft. telescope will exceed 2,000 tons, yet it will be capable of being steered with a precision of a fraction of a minute of arc and will also have a surface accurate to $\frac{1}{4}$ in. In addition to other small telescopes there is a horn 120 ft. long; the energy which this collects can be calculated from its dimensions and it is fixed so that it can observe the radio source in Cassiopeia each day, thus providing a standard calibration source in the sky available to many radio astronomers. A variety of receivers covering the wave-length range of 3·75–75 cm. is available, and three separate receivers working on different wave-lengths can be used simultaneously on the 85-ft. telescope. Besides being displayed on the chart of a pen recorder, the receiver outputs are digitized together with telescope position and time. Extensive use of digital computer techniques is already being made in analysing and reducing the observations.

Among the first observations made at the National Radio Astronomy Observatory were a study of the radio emissions from Jupiter and a survey of the central regions of the Galaxy at a wave-length of 3·75 cm. showing remarkable detailed structure. In addition, studies are being made of planetary nebulæ and ionized hydrogen clouds, supernovæ remnants and peculiar galaxies.

Three years were devoted to testing the seeing conditions at a number of places before Kitt Peak was chosen as the site for the National Observatory in March 1958. An agreement with the Papagos Indians (who call astronomers "the People with the Long Eyes") leased 200 acres to the Observatory, and construction of an 80-in. telescope is under way. This will have a coudé focus as well as prime and cassegrain, and will be equipped for direct photography, photoelectric photometry and spectroscopy. There is also a 36-in. telescope with a cassegrain spectrograph, and two 16-in. telescopes which will be used for bright star work. The University of Arizona is also moving a 36-in. telescope to the site.

On Kitt Peak there will be an optical workshop with some of the best testing facilities in the world, together with an office and laboratory building, dining hall and dormitory, houses and a shop. But the headquarters are adjacent to the University of Arizona in Tucson, and the staff and visiting astronomers will spend most of their time in the research offices there.

It has recently been decided to build a large solar telescope on Kitt Peak. The heliostat will have an 80-in. plane mirror feeding a 60-in., 300-ft. focal length concave mirror at the bottom of a tunnel 500 ft. long. The image of the Sun, 34 in. in diameter, will be reflected on to the slit of a large vacuum spectrograph. The outside of the building will be maintained at ambient temperature to prevent disturbing convection currents.

The National Observatory and the National Radio Astronomy Observatory will be valuable additions to the research facilities of universities which could not, alone, have provided them.

V. C. Reddish

BIOLOGICAL EFFECTS OF EXPOSURE TO INTENSE RADIATION

A UNIQUE and important experiment to determine the biological effects of acute and high-level radiation exposure has recently been completed under the auspices of the International Atomic Energy Agency at the Boris Kidrich Institute at Vinca, near Belgrade, Yugoslavia. Details of the experiment were given at a press conference held at Vinca on April 27 by the international team of scientists responsible for its organization and execution, and a brief report of the experiment and the conference is published in the *International Atomic Energy Agency Bulletin*, 2, No. 3, 3 (July 1960).

It will be recalled that on October 15, 1958, during an accident to the nuclear reactor *RB* at the Institute, six persons in the immediate vicinity of the unshielded

reactor were subjected to high doses of neutron- and gamma-radiation, and two others, farther away, received radiation doses in excess of the permissible level. After first-aid treatment at the Institute, they were transferred to the Centre for Professional Diseases in Belgrade. The six heavily irradiated patients were flown the following day to the Curie Hospital in Paris to receive special treatment by Dr. H. Jammet. One of the patients was cured by conventional treatment, including blood transfusions; but the other five, who had had blood-forming tissues in their bone marrow destroyed by the radiation, were given treatment involving the grafting of healthy bone marrow obtained from donors matched as closely as possible with the patients by detailed blood tests. This method of treatment had been tried before on an experimental basis but had not proved particularly successful. One of the five, presumably the one who had received the highest radiation dose, died before the treatment could take effect. The four others were gradually but successfully cured. A full report of the treatment has not yet been published. The success, however, represents a landmark in medical history and will intensify research in this field.

It was recognized that if the effects produced on the irradiated persons could be related to the exact doses of radiation they had received, valuable knowledge about the biological consequences of acute and high-level radiation exposure on a quantitative basis would be obtained. Although the Institute estimated that the irradiated persons received a total average whole-body dose of 683 rems of neutron- and gamma-radiation, these were not precise enough data, and accordingly the director-general of the International Atomic Energy Agency suggested to the Yugoslav authorities that a dosimetry experiment be conducted by an international team of scientists at Vinca. A formal agreement between the Agency and the Federal Nuclear Energy Commission of Yugoslavia was concluded on February 2, 1960, under which the Agency assumed responsibility for organizing and carrying out the experiment. Yugoslavia agreed to place the reactor and the laboratory at Vinca at the disposal of the Agency and to provide personnel, instruments and services. Experts from Saclay re-designed the control equipment of the reactor and were responsible for the reactor start-up and operation. The 6·5 tons of heavy water needed as moderator were provided free of charge by the U.K. Atomic Energy Authority and arrived at Vinca at the end of March. Twelve French experts, led by J. Weill and J. Furret, arrived with their special equipment from Saclay at the beginning of April, and an American team of seven health physicists from the Oak Ridge National Laboratory, together with their highly specialized equipment, including four 'phantoms' or plastic dummies of men filled with a salt solution, reached Vinca soon afterwards. The reactor first became critical on April 20. On April 22 it was operated at a power of 1 kW. and on April 25 at 5 kW. Simultaneous measurements of the neutron- and gamma-doses were made at points where the persons in the accident in October 1958 were located. The energy distribution of the radiation at these points was investigated, particularly the ratio of the various components.

At the press conference at Vinca, the scientists who had taken part in the experiment, and Dr. Jammet, were present. In response to questions, Dr. G. S. Hurst, of the American team, stated that there was one accident in the United States (that at Oak Ridge in June 1958) in which investigations comparable to those at Vinca were possible. It was not necessary to reconstruct the exact conditions of the incident of October 1958 for the dosimetry experiment, because the techniques of dosimetry did not depend on it—in any event it would have been impossible to do so because of certain unknown factors. It was, however, important to ensure that the configuration of the reactor room was exactly the same as during the accident.

Dr. Jammet explained during the press conference that not all the Yugoslav patients had received the same medical treatment, nor had the results been identical. The Curie Hospital had no fixed programme of treatment of radiation injury. There were always some unknown factors in specific cases and the problem was to make the treatment fit the specific needs of the case. Nevertheless, the experiment at Vinca was "another stone in the edifice of our research in the medical field".

AVIATION HYGIENE AND SANITATION

GENERAL provisions in the International Sanitary Regulations which were adopted by the Fourth World Health Assembly in 1951 require health administrations *inter alia* to ensure so far as is practicable that airports in their territories have at their disposal sufficient organization and equipment for the application of the regulations and are provided with supplies of pure drinking water and effective systems for the removal and safe disposal of excrement, refuse, waste water, condemned food, and other matter dangerous to health. Certain airports (designated as sanitary airports) should also be equipped with an organized medical service and facilities to combat infection. There are no specific provisions, however, on such matters as how pure drinking-water is to be supplied or an effective waste-disposal system organized, health administrations being left to make their own arrangements (*WHO Chronicle*, **14**, No. 6; June 1960).

The Assembly outlined several measures that should be taken, in conjunction with the International Civil Aviation Organization, to assist Member States to introduce high standards of hygiene and sanitation. Among them was the preparation of a guide to hygiene and sanitation in the operation of airports open to international traffic. This guide was prepared by a World Health Organization Committee on Hygiene and Sanitation in Aviation. It makes specific recommendations on water, food, and waste. All water for drinking and other personal use by crews and passengers, whether in the air or on the ground, should be free from chemical substances and micro-organisms that might cause illness in any form, and should not only be safe but also attractive,

lacking turbidity, colour and disagreeable taste or odour. Preferably, it should come from well-operated public supply systems conforming with acceptable standards. Samples should be taken at least once weekly from representative points, and the results of bacteriological analysis reported to the airport health and operating authorities and used to determine whether the airport water meets the bacteriological standard, and, if not, what corrective measures are required. The water supply should be inspected by a competent sanitary engineer at least once a year and whenever changes are made in the system that might affect the quality of the water.

If water must be drawn from a supply of inferior or unknown quality for use by crews or passengers, it should be given sufficient treatment to destroy cysts of *Entamoeba hystolytica* and the virus of infectious hepatitis; all the other disease organisms generally found in contaminated water will be destroyed by this treatment.

Food should be of good sanitary quality, placed in refrigerators as necessary, and kept scrupulously clean. All meat and fish should be thoroughly cooked before serving. In tropical countries infection with *Ascaris* is widespread, so that raw foods such as green salads and chopped raw vegetables should be avoided. All food should be carefully handled and protected from contamination by insects and other animals, soiled hands, and dirty surfaces. To prevent chemical poisoning, vessels and utensils containing such elements as cadmium and lead should be avoided, the strictest precautions observed regarding the use of poisons around food areas, and only food of the highest quality purchased. Because of the special hazards of foods like cream, milk and milk products, cracked eggs (especially duck's), 'shellfish', gravies and meat salads and dressings, preference should be given to plain, simple foods requiring the minimum of handling. Raw oysters and other shellfish should not be used unless it is certain that they are fresh, uncontaminated, and come from clean areas. Milk and milk products should be sterilized and kept in bottles; and the opened bottles should remain in refrigerators.

Special cleansing and germicidal treatment should be given to dishes and utensils before use.

The guide contains a detailed description of the handling and disposal of toilet wastes, sewage, air-sickness containers, and refuse, at airports and on the aircraft. Particular care should be taken to avoid spillage or open exposure, since this leads to fly and rat infestations. If practicable, the final disposal of garbage should be at a point remote from the airport; if not, it can be burned, buried, or ground and discharged into a sewer; open pits or dumps are unsatisfactory because of flies and rats. On aircraft, the provision of watertight receptables, easily cleanable floorings, soil-cans, tanks and wash-basins is important.

Rooms used by crews and passengers in transit at international airports should be effectively mosquito-proofed in areas where mosquitoes and flies are prevalent. Every day, any mosquitoes that may have gained entrance should be destroyed. Walls and ceilings, particularly in sleeping quarters, should be sprayed regularly with residual insecticide, in accordance with the recommendations of the WHO Expert Committee on Insecticides.

To carry out this work, airports must have adequate staff, equipment and premises. The adequacy and efficiency of ground installations are of interest and concern to many groups; to increase efficiency and co-operation, committees, such as national and local facilitation committees, should review procedures and practices and study possible improvements.

CENTENARY CELEBRATIONS OF W. M. BAYLISS AND J. S. HALDANE IN MOSCOW

A JOINT meeting of the U.S.S.R.–Great Britain Society and the Moscow Physiological Society was recently held at the Friendship House in Moscow. The meeting was devoted to the centenary of the births of W. M. Bayliss and J. S. Haldane.

The meeting was opened by Prof. V. Parin, president of the Physiological Society. Prof. K. Koshtoyants, a prominent Soviet physiologist and head of the Department of Physiology of the Moscow State University, discussed the life and research work of Prof. Bayliss. "Bayliss's name is one of the brightest in the history of world physiology", he said. Among other things, Prof. Koshtoyants said that soon after the Civil War a State Publishing House was set up in the Soviet Union. It planned the publication of a number of major works of world science, and one of the first in the list of these works was Bayliss's famous book "Principles of General Physiology". In 1927, Russian translations of two monographs by Bayliss were published. Prof. Koshtoyants went on to speak of the profound mutual interest in, and respect for, each other of Bayliss and the equally great Russian physiologist Ivan Pavlov. It so happened that Pavlov in Russia and Bayliss and Starling in Britain did parallel research on the regulation of the activity of the pancreas, but came to opposite conclusions. According to Pavlov, the gland produced its secretion as a result of nervous signals, whereas according to Bayliss the gland was made to function by an active chemical agent contained in the blood. A thorough joint investigation of the experiments showed that the Russian and the British physiologists were both right, and as a result a comprehensive modern conception of the regulation of pancreatic secretion was formed.

Prof. L. Shik then spoke of the research work done by J. S. Haldane. As a result of Haldane's researches, he said, basic conceptions were formed of the physiology of respiration. Haldane's experiments were distinguished for their remarkable outward simplicity, which was actually the result of a good deal of thought and precise preliminary calculations. Unlike many representatives of the so-called 'pure science', Haldane was interested in the problems of applied physiology, and his research on the physiology of divers and airmen provided scientific grounds for modern practical measures ensuring man's safety in the conditions of changing barometric pressure. Many of his experiments, some of which were quite

dangerous, he conducted on himself. No wonder that when the well-known Russian physiologist, A. Samoilov, urged young research workers to investigate directly the human organism, he always referred to Haldane's successful experiments.

Some of the members of the U.S.S.R.–Britain Society prepared for the jubilee meeting an exhibit with the portraits of Bayliss and Haldane and a display of Russian translations of their books.

D. A. Sakharov

IS HYPERCONJUGATION NECESSARY?

MULLIKEN, Reike and Brown[1] proposed a new type of conjugation, 'hyperconjugation', based on the fact that the heat of hydrogenation of an olefin is appreciably decreased by the presence of alkyl substituents on the unsaturated carbon atom. A necessary effect of hyperconjugation would be to change the lengths of the C—H bond in the alkyl group and the C—C bond adjacent to a double or triple bond. The C—Me distance in toluene[2] is 1·51 Å. and in methyl acetylene and acetonitrile[3] it is 1·46 Å., compared with the normal C—C distance of 1·54 Å. in ethane. It was pointed out that part of these bond shortenings was due to the change in the atomic radius from tetrahedral to trigonal or digonal carbon and about one-half of the observed shortening was due to hyperconjugation[4].

Hyperconjugation was considered to be responsible for the dipole moment of alkyl benzenes. The ionization potentials of methylated ethylenes[4,5] and the absorption spectra[5,6] were also given as evidence for hyperconjugation. The view that C—H hyperconjugation is the predominant mode of electron release by alkyl substituents when attached to an electron-demanding system was originally proposed by Baker and Nathan as an explanation of certain rate and equilibrium data. The Baker–Nathan effect was in the order Me > Et > *i*-Pr > *t*-Bu, which is exactly the reverse of what one would expect if the electron release were by the inductive mechanism.

Although a great deal of physical and chemical evidence has been accumulated in support of the idea of hyperconjugation, recently there have been a number of observations which cannot be explained in a simple fashion by hyperconjugation, and the situation has become controversial.

Recent molecular structure investigations[3,7] in several laboratories, on derivatives of acetonitrile and methyl acetylene, have conclusively shown that the length of a carbon–carbon single bond adjacent to a triple bond (that is, a bond between *sp* and sp^3 carbon atoms) is always close to 1·46 Å., independent of the substituents on the sp^3 carbon. So it is concluded that the shortenings in the C—C bonds between *sp* and sp^3 carbon atoms (0·08 Å.) or sp^2 and sp^3 carbon atoms (0·04 Å.) may not result from any kind of conjugation, and that it is likely that these bond contractions are entirely due to the changes in the covalent radius of carbon with hybridization. The bond-lengths in relation to hyperconjugation have been discussed in fair detail by Sutton[8]. The evidence for hyperconjugation from calorimetric data is no longer valid, since the change in the C—C bond energies with bond-lengths (that is, with hybridization) was not taken into account in these calculations. So the effects attributed to hyperconjugation of the alkyl groups may equally be due to the introduction of a stronger C—C bond. In general, the stabilization of an unsaturated system by alkyl substitution has been found to be essentially independent of the nature of the alkyl group and cannot be accounted for in terms of steric effects[9].

The evidence for hyperconjugation from dipole moment data also needs to be re-interpreted. Since the electronegativity of an *sp* or sp^2 carbon is different from that of an sp^3 carbon, the resulting bonds (between any two differently hybridized carbon atoms) should have different polarities. In fact, Petro[10] has reported excellent agreement between the observed and calculated dipole moments of C—C bonds between two differently hybridized carbon atoms. He concludes that hyperconjugation structures do not explain the non-zero dipole moment of toluene. Recently, Brown[11] has studied the dipole moments of alkyl benzenes and the results are not conclusive.

The earlier evidence for hyperconjugation on the basis of ionization potentials and absorption spectra was not unambiguous because these deal with both the electronic ground-states and the excited states of molecules. The ionization potentials of the alkyl groups are actually found to be in the inductive order[12]. However, a simple molecular orbital treatment by Streitwieser and Nair[13] seems to indicate that the methyl group can be treated as a 'heteroatom' donating two electrons to the π-system. The evidence from absorption spectra has met with a number of objections[14–16]. An explanation based on steric hindrance to solvation of the electron-deficient sites in the vicinity of the alkyl substituent has been suggested.

In general, it may be said that the effect of alkyl groups on the near ultra-violet absorption spectra of aromatic compounds is not consistent. A recent study of the $n \rightarrow \pi^*$ transitions of aliphatic ketones and nitro compounds in different types of solvents by Rao *et al.*[17] seems to show the predominant role of resonance interaction of the alkyl groups in the excited states of these molecules. An earlier investigation by Rao, Goldman and Lurie[18] of the infra-red carbonyl frequencies in aliphatic ketones had indicated that mainly inductive effects were important in the ground-states of these molecules.

The chemical evidence for hyperconjugation has been a subject of controversy. The Baker–Nathan order Me > Et > *i*-Pr > *t*-Bu, has been found in a number of reactions and has been explained in terms of the C—H hyperconjugation[19]. The importance of C—C hyperconjugation in the *t*-butyl group has also been pointed out[20]. The relative importance of C—H and C—C hyperconjugation in aliphatic and aromatic series has been evaluated by the application of the linear inductive energy relationship[21,22]. McCaulay and Lien[23] have shown that C—H hyperconjugation must be an important mode of electron release in the methyl-substituted aromatic cations. De la Mare[24] has discussed the possibility of OH and NH hyperconjugation. Shiner[25], Lewis[26] and Taft and co-workers[27] have shown that hypercon-

jugation is one of the important sources of secondary isotope effects. Shiner[25] considers three possible effects on hyperconjugation: steric, substituent and solvent effects. Brown[26] has recently discussed the relative importance of the dispersion and induction forces in alkyl benzenes. Berliner[19] is of the opinion that although the effect of the alkyl groups in individual reactions can be interpreted in different ways (based on solvent or steric or inductive effects), hyperconjugation appears to be the most satisfactory general explanation for the behaviour of remote alkyl groups on the benzene ring where the Baker–Nathan order is observed.

The information from chemical reactivity regarding the possible role of hyperconjugation is not consistent, and does not distinguish such a contribution in the ground-state from that in the transition state of the molecule. Further, there is no clear evidence for any marked degree of hyperconjugation from physical measurements on the ground-states of molecules. These arguments have been forcefully expressed by Dewar and Schmeising[29], who have developed a new method for calculating bond-lengths and resonance energies for conjugated systems taking the σ-bond compression into consideration. They argue that if the π-electron resonance is unimportant in ordinary conjugation (as in the case of butadiene), it should be much more so in hyperconjugation. But Mulliken[20] has found it desirable explicitly to classify conjugation and hyperconjugation into two types: isovalent and sacrificial (ordinary). He also suggests further classification of isovalent conjugation and isovalent hyperconjugation into sub-types. Isovalent hyperconjugation has been suggested as being more important than ordinary hyperconjugation. The Baker–Nathan effect has been described as differential hyperconjugation. Apparently there are no theoretical reasons for expecting differences between C—C and C—H hyperconjugation[30].

In my view, the present status of hyperconjugation may be summarized as follows: while the contribution from hyperconjugation seems unimportant in the electronic ground-states of molecules, it may be prominent in the excited or the transition states of molecules.

I have benefited greatly from stimulating discussions with colleagues in Purdue University and the University of California. Many of the papers referred to as appearing in *Tetrahedron* were published in a special issue of that journal containing the proceedings of a Conference on Hyperconjugation held at Indiana University in June 1958.

C. N. R. Rao*

* Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore.

[1] Mulliken, R. S., Reike, C. A., and Brown, W. G., *J. Amer. Chem. Soc.*, **63**, 41 (1941).
[2] Kiedel, F. A., and Bauer, S. H., *J. Chem. Phys.*, **25**, 1218 (1956).
[3] Zeil, W., and Pfrommer, J. F., *Z. Elektrochem.*, **61**, 938 (1957).
[4] Coulson, C. A., "Valence" (Clarendon Press, Oxford, 1952).
[5] Baker, J. W., "Hyperconjugation" (Oxford Univ. Press, 1953).
[6] Matsen, F. A., Robertson, W. W., and Chuoke, R. L., *Chem. Revs.*, **41**, 273 (1947).
[7] Muller, N., and Pritchard, D. E., *J. Amer. Chem. Soc.*, **80**, 3483 (1958).
[8] Sutton, L. E., *Tetrahedron*, **5**, 118 (1959).
[9] Turner, R. B., *Tetrahedron*, **5**, 127 (1959).
[10] Petro, A. J., *J. Amer. Chem. Soc.*, **80**, 4230 (1958).
[11] Brown, T. L., *J. Amer. Chem. Soc.*, **81**, 3232 (1959).
[12] Hammond, V. J., Price, W. C., Teegan, J. P., and Walsh, A. D., *Discuss. Farad. Soc.*, **9**, 53 (1950).
[13] Streitwieser, jun., A., and Nair, P. M., *Tetrahedron*, **5**, 149 (1959).
[14] Platt, J. R., and Klevens, H. B., *Chem. Revs.*, **41**, 301 (1947).
[15] Bowden, K., and Braude, E. A., *J. Chem. Soc.*, 1068 (1952).
[16] Schubert, W. M., Craven, J. M., Minton, R. G., and Murphy, R. B., *Tetrahedron*, **5**, 194 (1959).
[17] Rao, C. N. R., *et al.*, *Tetrahedron Letters*, 2 (1960); *Canad. J. Chem. and Chem. Indust.* (in the press).
[18] Rao, C. N. R., Goldman, G. K., and Lurie, C., *J. Phys. Chem.*, **63**, 1311 (1959).
[19] Berliner, E., *Tetrahedron*, **5**, 202 (1959).
[20] Brown, H. C., Brady, J. D., Grayson, M., and Bonner, W. H., *J. Amer. Chem. Soc.*, **79**, 1897 (1957).
[21] Taft, jun., R. W., and Lewis, I. C., *Tetrahedron*, **5**, 210 (1959).
[22] Baker, J. W., *Tetrahedron*, **5**, 135 (1959).
[23] McCaulay, D. C., and Lien, A. P., *Tetrahedron*, **5**, 186 (1959).
[24] De la Mare, P. B. D., *Tetrahedron*, **5**, 107 (1959).
[25] Shiner, jun., V. J., *Tetrahedron*, **5**, 243 (1939).
[26] Lewis, E. S., *Tetrahedron*, **5**, 143 (1959).
[27] Love, P., Taft, jun., R. W., and Wartik, T., *Tetrahedron*, **5**, 116 (1959).
[28] Brown, T. L., *J. Amer. Chem. Soc.*, **81**, 3229 (1959).
[29] Dewar, M. J. S., and Schmeising, H. N., *Tetrahedron*, **5**, 166 (1959).
[30] Mulliken, R. S., *Tetrahedron*, **5**, 253 (1959).

THE 'GBR EXPERIMENT': A TRANS-ATLANTIC FREQUENCY COMPARISON BETWEEN CÆSIUM-CONTROLLED OSCILLATORS

By J. A. PIERCE
Cruft Laboratory, Harvard University, Cambridge, Mass.

G. M. R. WINKLER
U.S. Army Signal Research and Development Laboratory, Ft. Monmouth, New Jersey

AND

R. L. CORKE
Post Office Research Branch, Dollis Hill, London, N.W.2

FOR several years the temporal variation of phase, and hence the frequency, of a 16-kc./s. signal from Rugby, England, has been observed at Cambridge, Massachusetts[1,2]. During this period the transmission has been controlled by an Essen ring crystal oscillator and the reference at Cambridge has been one of several crystals or, since early 1958, an Atomichron.

The problem of resolving the observed fluctuations of phase into components caused on the propagation path and those produced by one or both oscillators has been attacked by choosing short periods (usually 4 or 5 days) when operation seemed especially stable. For these short lengths of time, internal evidence seemed to indicate that the oscillators were behaving in a consistent way and the observed deviations were taken as representing primarily variations in transmission time. The results of this somewhat dubious procedure indicated standard deviations of phase of about 1 or 2 μsec. When observations were extended over 24 hr., the corresponding precision of frequency measurement should have been about two parts in 10^{11}.

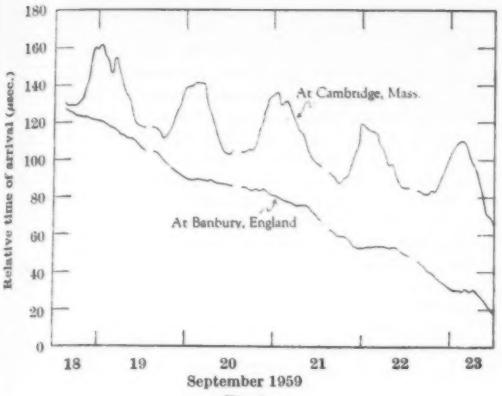


Fig. 1

Unfortunately, all long-term frequency comparisons have yielded standard deviations of 2 or 3 parts in 10^{10}. The source of this discrepancy of ten times in precision has been assumed to be frequency uncertainty in crystal oscillators. Proof, however, was lacking, and the corollary assumption that the long-distance transmission is more stable than very good oscillators has not been easily accepted in some quarters.

In this dilemma a simple experiment was performed during September and October 1959. A photographic record of the phase of the Rugby signal was made at Banbury, Oxfordshire, England, where transmission vagaries on the 24-mile path from Rugby could be assumed to be unimportant. This record was, in every way, exactly similar to one simultaneously made in Cambridge, Massachusetts. The Banbury record was referred to Atomichron No. 111 and the Cambridge record was made in terms of Atomichron No. 112. Thus, by subtraction, the effects of the fluctuations of the crystal controlling the transmitter could be removed.

An example of the observed phase (or time of arrival) variations is given in Fig. 1. The Cambridge data show the normal day-to-night propagational variation of some 34 μsec. Both curves indicate some crystal oscillator variation, and the gradual divergence between the curves indicates that there was some frequency difference between the two Atomichrons.

It was hoped that this experiment would yield at least a month of continuous operation at both receivers. This hope was not realized, because of a number of minor interruptions which caused loss of continuity of phase at one point or the other. There were, however, two periods of ten days or so when operation was entirely satisfactory. During the first of these, the difference in phase between Cambridge and Banbury is shown in Fig. 2. Here it can scarcely be denied that the frequency of one of the Atomichrons was shifting with respect to the other, as shown by the gradual change in slope of the curve, when diurnal variation is removed. In spite of this effect, excellent day-to-day consistency is observed.

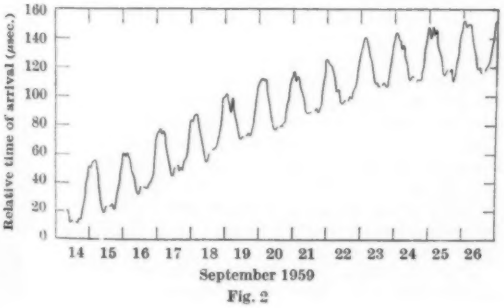


Fig. 2

The other period of satisfactory operation gave the difference record of Fig. 3. In this case the sense of the frequency difference between the Atomichrons had reversed, but the difference seems small and quite stable.

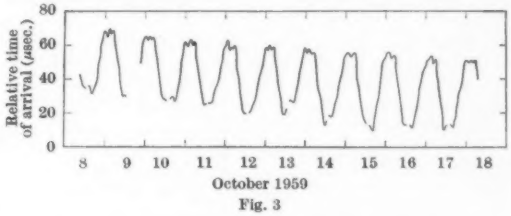


Fig. 3

In a record of this sort, any chord has a slope that is a measure of the frequency difference for the interval between its end points. The scale of the figures exhibited is such that even the steep slopes at sunrise and sunset represent frequency variations of only about two parts in 10^9. To remove the diurnal variation it is simple to take measurements over 24 hr. or multiples thereof. This might be done, for example, from noon to noon. In this article, however, each day's record (from 15h. U.T. to 13h. U.T. on the following day) is reduced to a single value by averaging together the 23-hourly values.

The time-of-arrival values thus obtained are shown in the second column of Table 1. Values are missing for October 8–10 (although some are shown in Fig. 3)

Table 1. Frequency Differences between Atomichrons

Date 1959	No. 111 relative to 112			No. 111 relative to 117	
	Mean relative phase (in μs.)	Frequency difference in parts (in 10^{10})	Deviation of frequency difference* in parts (in 10^{10})	Frequency difference† in parts (in 10^{10})	Deviation of frequency difference* in parts (in 10^{10})
Sept. 14	32·7			0·7	+0·4
15	41·3	−1·0	+0·5	0·2	−0·1
16	56·0	−1·7	−0·3	0·2	−0·1
17	66·7	−1·2	+0·1	0·2	−0·1
18	80·7	−1·6	−0·4	0·3	0·0
19	90·5	−1·1	0·0	0·3	−0·1
20	96·9	−0·7	+0·3	0·2	−0·2
21	104·3	−0·9	+0·1	0·3	−0·1
22	117·1	−1·5	−0·6	0·3	−0·1
23	123·3	−0·7	+0·1	0·4	0·0
24	128·0	−0·5	+0·2	0·4	0·0
25	132·0	−0·5	+0·1	0·8	+0·4
26					
Oct. 10	42·6			1·2	−0·1
11	41·2	+0·2	−0·1	1·2	0·0
12	38·7	+0·3	+0·1	1·3	+0·2
13	39·1	0·0	−0·3	1·1	+0·1
14	36·1	+0·3	+0·1	0·8	−0·1
15	33·8	+0·3	+0·1		
16	32·5	+0·2	0·0		
17					
Mean‡		−0·4		0·6	
Root mean square‡		0·8		0·7	
Standard deviation*			0·26		0·17

* With respect to best straight lines.
† Sense of difference unknown, but taken as positive.
‡ Including a few values for intervening and subsequent dates.

because of a recording failure on October 9 which lost some of the 23 hourly values on that day. Column 3 of Table 1 gives the frequency difference derived from the successive phase values of column 2. Because of the curvature shown in Fig. 2, which may have been caused by the incipient failure of the klystron in Atomichron 112 that occurred on September 27, a progressive change in frequency has been assumed. The deviations of column 4 of Table 1, therefore, imply a linear change in frequency or a parabolic change in time of arrival. It will be seen that the frequencies are consistent to a few parts in 10^{11}.

At the time of these observations a second Atomichron, No. 117, was also operating at Banbury. It was not involved in the measurements hitherto discussed, but beats were counted between the two local outputs. These were not continuously reported, and the sense of the frequency difference was not determined. The values shown in column 5 of Table 1 have been assumed positive, for computation of the deviations in column 6.

It is perhaps significant that the r.m.s. differences at the bottom of Table 1 are so similar in magnitude. This value seems to represent the precision with which two Atomichrons can be expected to produce the same frequency.

The standard deviations show the accuracy with which the Rugby signal can be measured, in Cambridge and in Banbury, in an interval of 24 hr. This precision is, at least on the average, inversely proportional to the duration of measurement. Although there is no firm reason to assume that the frequency fluctuations of three Atomichrons are identical, the similarity between the two standard deviations is striking. It appears, as suggested initially, that the actual fluctuations in transmission time are of the order of two microseconds and the corresponding contribution to the errors of trans-Atlantic frequency measurement is about two parts in 10^{11}, for a duration of measurement of 24 hr.

Acknowledgment is made to the Engineer-in-Chief of the British Post Office for the facilities made available at the Post Office Radio Measuring Station, Banbury, Oxfordshire, England. We thank Mr. W. N. Genna and the staff at Banbury for their assistance.

[1] Pierce, J. A., Mitchell, H. T., and Essen, L., *Nature*, **174**, 922 (1954).
[2] Pierce, J. A., *Proc. Inst. Rad. Eng.*, **45**, 794 (1957).

CHEMICAL AND PHYSICAL PROPERTIES OF THE SMALL DEOXYRIBONUCLEIC ACID COMPONENT OF CRYSTALLINE CYTOCHROME B_2

By Dr. M. D. MONTAGUE and Prof. R. K. MORTON
Department of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide

THE recent report by Kit[1] concerning the fractionation of deoxyribonucleic acid on columns of 'Ecteola-cellulose' prompts us to report our experience with the deoxyribonucleic acid component associated with crystalline cytochrome b_2 from baker's yeast.

Cytochrome b_2 crystallized by the procedure of Appleby and Morton[2] is a flavohæmoprotein[2,3] containing a deoxyribonucleic acid component[4] and having L(+)-lactate dehydrogenase activity. The deoxyribonucleic acid component was dissociated from the enzyme, which had been crystallized three times, by anaerobic dialysis at *p*H 8·0 against 0·7 saturated ammonium sulphate solution[4] containing 0·15 *M* sodium lactate and 10^{-4} *M* ethylenediamine tetraacetate. The solution of nucleic acid was dialysed against several changes of 0·01 *M* potassium phosphate buffer, *p*H 7·0 and about 500 μgm. of the nucleic acid were loaded on to a column (3·5 × 1 cm.) of 'Ecteola-cellulose' (type 20, Brown Co., Berlin, New Hampshire). A gradient of sodium chloride in 0·01 *M* potassium phosphate buffer, *p*H 7·0, was used to elute the components. Approximately 85 per cent of the material absorbing at 260 mμ was eluted as a single peak between 0·5 and 0·7 *M* sodium chloride. Re-chromatography on the same column using a less-steep gradient of sodium chloride gave a single peak between the same limits of salt concentration. Alternate dialysis and freeze-drying was used to concentrate the material and to avoid possible denaturation due to high concentrations of salt. Table 1 shows the optical properties of the deoxyribonucleic acid from cytochrome b_2.

Table 1. Optical Properties of the Deoxyribonucleic Acid Component of Crystalline Cytochrome b_2

	$\lambda_{max.}$ (mμ)	ε(P) (max.)	$\lambda_{min.}$ (mμ)	ε(P) (min.)	$E250\,m\mu / E260\,m\mu$	$E280\,m\mu / E260\,m\mu$	$E290\,m\mu / E260\,m\mu$
In sodium acetate (0·01 *M*) *p*H 7·0	260	6,600	233	2,620	0·82	0·51	0·19
In NaOH (0·05 *N*)	260	7,800	235	4,300	0·80	0·46	0·12
In HCl (0·01 *N*)	260	8,650	232	2,740	0·76	0·56	0·27

After removing most of the ribonucleic acid by fractionation with sodium chloride, Kirby's[5] procedure was used to isolate the bulk deoxyribonucleic acid of yeast extracted from the same type of baker's yeast as used for preparation of the cytochrome b_2. The bulk deoxyribonucleic acid was chromatographed on the same column as was used for the deoxyribonucleic acid from cytochrome b_2. A component (fraction 6–7), comprising 15 per cent of the total material absorbing at 260 mμ, was eluted with 0·6–0·7 *M* sodium chloride. It will be noted that the deoxyribonucleic acid component from cytochrome b_2 was eluted between 0·5 and 0·7 *M* sodium chloride under similar conditions.

Table 2 shows the base compositions of the deoxyribonucleic acid from cytochrome b_2, of the bulk

Table 2. Base Compositions of Deoxyribonucleic Acid Preparations from Baker's Yeast*

Preparation	Guanine	Cytosine	Adenine	Thymine	(Adenine + Thymine) (Guanine + Cytosine)
Bulk DNA	17·4 17·3	16·6 16·2	32·2 32·6	33·8 33·9	1·9 2·0
Fraction 6–7 from bulk DNA†	17·5 16·6	14·9 14·8	33·5 33·9	34·1 34·7	2·2 2·2
DNA from cytochrome b_2	14·5 15·1 15·0	14·0 14·4 14·5	33·7 33·9 34·7	37·8 36·6 35·8	2·5‡ 2·4 2·4

* The procedures are described elsewhere (refs. 4, 6). The results are expressed as a percentage of total bases; the recovery was 92–96 per cent in each case.
† Fraction eluted between 0·6 and 0·7 *M* sodium chloride from 'Ecteola-cellulose' column.
‡ Appleby and Morton (ref. 4) found a base ratio of 2·6 from analyses on whole enzyme.

deoxyribonucleic acid from yeast, and of fraction 6–7 from this preparation.

There was a 30 per cent increase in extinction at 260 mμ after digestion of 250 μgm./ml. of the deoxyribonucleic acid from cytochrome b_2 in 0·02 *M* magnesium chloride with 2 μgm./ml. of crystalline pancreatic deoxyribonuclease at *p*H 7·0; there was no initial lag in the reaction, in contrast to the reaction of the same enzyme with the bulk deoxyribonucleic acid of the yeast. The component from cytochrome b_2 was not degraded by snake venom phosphodiesterase.

Work on the end-group of the deoxyribonucleic acid from cytochrome b_2 has been carried out, the deoxyribonucleoside components at the ends of the chains terminated by 5′-phosphate groups being released by enzymic degradation. All incubations were carried out at room temperature (about 20° C.). A solution containing 270 μgm. of the nucleic acid from cytochrome b_2 in 0·02 *M* magnesium chloride at *p*H 7 was incubated for 16 hr. with potato phosphomonoesterase (free of phosphodiesterase activity) and then heated at 100° C. for 2 min. to destroy the phosphatase activity. It was then digested with crystalline pancreatic deoxyribonuclease for 10 hr. The solution was adjusted to *p*H 8·9 and then incubated for 12 hr. with snake-venom phosphodiesterase (free of phosphomonoesterase activity). The solution was then heated at 100° C. for 1 min., and the clear supernatant obtained after centrifuging was chromatographed on Whatman paper using water-saturated *n*-butanol (100 ml.)–ammonia (1 ml. of solution, specific gravity 0·880). Two components, with R_F values of 0·22 and 0·30, were identified by their ultraviolet absorption spectra as derivatives of guanine and cytosine respectively. Neither adenine nor thymine derivatives were detected. The amounts of nucleosides isolated were too small to determine the quantities of each present with high accuracy, but from the estimates of the amounts released, the molecular weight of the nucleic acid is 10,000–12,000.

The sedimentation coefficient of the deoxyribonucleic acid from cytochrome b_2 was measured using a 'Spinco' model *E* ultracentrifuge fitted with ultraviolet optics. At a concentration of approximately 0·003 per cent of nucleic acid in 0·01 *M* potassium phosphate, 0·078 *M* sodium chloride at *p*H 7·0 (Γ/2, 0·10) values for $S_{20,w}$ of 5·6–5·9 svedbergs were obtained. A preliminary diffusion experiment using the ultracentrifuge with a synthetic boundary cell indicated that the molecular weight of the nucleic acid is 10,000–20,000. No marked heterogeneity of the sedimenting nucleic acid was observed. We are grateful to Mr. J. McD. Armstrong for these measurements.

Since nucleotide and hæm analyses of crystalline cytochrome b_2 indicate that the minimum molecular weight of the deoxyribonucleic acid component of the enzyme is about 5,000[4], the end-group assays and work on sedimentation establish that the actual molecular weight in solution is approximately 10,000.

The work by Kit[1] on the fractionation of various deoxyribonucleic acid preparations on 'Ecteola-cellulose' (type 20, Brown Co.) has shown that the elution pattern depends markedly on the method of isolation of the nucleic acid. Much of the deoxyribonucleic acid of low molecular weight eluted by neutral salt solution appears to result from the degradation of larger molecules by a number of mechanisms including heat denaturation, action of deoxyribonuclease and mechanical cleavage when the cells are ruptured. Nevertheless, if precautions are taken to eliminate or reduce these degradative processes, the deoxyribonucleic acid still contains material of low molecular weight, and so far no bulk deoxyribonucleic acid has been isolated which does not contain at least 5 per cent of small components. Thus the possibility that the cell normally contains a complement of deoxyribonucleic acid of low molecular weight has still to be considered.

The function of the deoxyribonucleic acid of crystalline cytochrome b_2 is not known. It is not necessary for enzymic activity[4]. Under conditions of low ionic strength, the enzyme does not crystallize in the absence of this component[4] and it may be that the nucleic acid provides the necessary rigidity for crystallization of this protein. It is significant that the ratio of (adenine + thymine) to (guanine + cytosine) for the nucleic acid of cytochrome b_2 is consistently 2·4–2·6, as compared with the value of 1·95 for the bulk deoxyribonucleic acid of the same yeast (Table 2).

The constancy of composition indicates that the deoxyribonucleic acid of crystalline cytochrome b_2 is a specific component of the yeast cell rather than a small molecule formed by random degradation of larger nucleic acid molecules; in the latter case, the base composition of the nucleic acid would be expected to vary among different preparations. The base ratio found in the component (fraction 6–7) from the bulk deoxyribonucleic acid of yeast (Table 2) emphasizes this conclusion.

The constancy in behaviour of different preparations during chromatography on 'Ecteola-cellulose', the sedimentation and diffusion characteristics, and the end-group assay all indicate that the deoxyribonucleic acid component from crystalline cytochrome b_2 is probably a homogeneous material of low molecular weight (10,000). It therefore appears to represent a suitable material with which to investigate the sequence of nucleotides in naturally occurring deoxyribonucleic acid. A detailed account of these investigations and of further work with this material will be reported elsewhere.

1 Kit, S., *Arch. Biochem. Biophys.*, **87**, 318 (1960).
2 Appleby, C. A., and Morton, R. K., *Nature*, **173**, 749 (1954); *Biochem. J.*, **71**, 492 (1959).
3 Appleby, C. A., and Morton, R. K., *Biochem. J.*, **73**, 539 (1959).
4 Appleby, C. A., and Morton, R. K., *Biochem. J.*, **75**, 258 (1960).
5 Kirby, K. S., *Biochem. J.*, **66**, 495 (1957).
6 Wyatt, G. R., in "The Nucleic Acids", edit. by Chargaff, E., and Davidson, J. N., 1, 243 (Academic Press, New York, 1955).

MECHANISM OF STARCH BIOSYNTHESIS

By Dr. MARIA A. RONGINE DE FEKETE*, Dr. LUIS F. LELOIR and Dr. CARLOS E. CARDINI
Instituto de Investigaciones Bioquímicas "Fundación Campomar", and Facultad de Ciencias Exactas y Naturales, Obligado 2490, Buenos Aires

SINCE the classical work of Hanes[1] some workers consider that starch synthesis *in vivo* is catalysed by phosphorylase. Nevertheless, others have raised doubts on this hypothesis. Thus Ewart *et al.*[2], from measurements of the ratio of inorganic phosphate to glucose-1-phosphate, concluded that: "phosphorylase is not involved in the synthesis of starch . . . but the role of phosphorylase in the normal metabolic breakdown . . . is not questioned" (cf. also Rowan and Turner[3]). Furthermore, Stocking[4] reported that starch synthesis in leaves is initiated in the chloroplasts, where phosphorylase could not be detected.

The problem of starch synthesis is similar to that of glycogen synthesis in animal tissues. The synthetic role of phosphorylase *in vivo* has been challenged on the basis of the following facts: (*a*) the unfavourable ratio of inorganic phosphate to glucose-1-phosphate in tissues; (*b*) agents which increase the concentration of phosphorylase, such as epinephrine and glucagon, produce glycogen breakdown[5]; and (*c*) in certain diseases phosphorylase is absent in the muscles, although normal or increased amounts of glycogen are present[6].

If these facts are sufficient to rule out phosphorylase as the enzyme responsible for glycogen synthesis, the only enzyme which can take its place, so far as we know, is glycogen synthetase[7], which catalyses the transfer of glucose from uridine diphosphate glucose to glycogen forming a new α-1 : 4 linkage. On the other hand, it is known that uridine diphosphate glucose acts as glucose donor in plants. Thus enzymes catalysing the synthesis of sucrose[8], sucrose phosphate[9], and callose[10] have been described. The latter is a β-1 : 3 glucan[11] discovered many years ago and believed to have a role in the physiology of sieve tubes and in wound reaction in plants[12].

All this suggests that uridine diphosphate glucose should be involved in starch synthesis. Although incubation of radioactive uridine diphosphate glucose with different plant materials usually resulted in the incorporation of radioactivity in the polysaccharide fraction, the product formed was insoluble in hot water, was not hydrolysed by β-amylase and was presumably callose.

Since glycogen synthetase has been found to be strongly adsorbed on glycogen (ref. 7*c*), we have looked for a starch-synthesizing enzyme in starch granules. In preliminary experiments, radioactive uridine diphosphate glucose was incubated with freshly prepared potato starch and it was found that there was some incorporation of radioactivity in the fraction which became soluble by the subsequent action of α-amylase. More active preparations were obtained from beans as follows. Freshly harvested immature dwarf string beans (*Phaseolus vulgaris* var. Bountiful) were used. The cotyledons and the embryos were ground in a mortar with two volumes of water. The suspension was filtered through cheesecloth and centrifuged 5 min. at 3,000 rev./min. The precipitate was resuspended in several volumes of water and recentrifuged three times. The white precipitate was then suspended in four volumes of acetone at − 15° C., centrifuged at 0° C.; this procedure was repeated three times, after which the precipitate was dried *in vacuo*. This preparation could be stored for months at − 15° C. with no decrease in activity.

The bean starch fraction (2 mgm. containing 6 μgm. of protein) was incubated at 37° C. for 3 hr. with 0·21 μmole of uridine diphosphate glucose containing 7,850 counts/min. of carbon-14 in the glucose moiety, 2 μmoles of glycine buffer of *p*H 8·4, 0·05 μmole of ethylenediamine tetraacetate in a final volume of 15 μl. After incubation, 0·5 ml. of 80 per cent ethanol was added. The soluble fraction was spotted on paper and chromatographed with ethanol–ammonium acetate of *p*H 7·5 (ref. 15). The uridine diphosphate and uridine diphosphate glucose spots were eluted from the paper and the absorbancy at 260 mμ, radioactivity and uridine diphosphate were measured. The insoluble fraction was washed with ethanol and counted. Blanks without and with uridine diphosphate glucose added after incubation were run at the same time. Furthermore, it was found that the bean starch fraction did not destroy added uridine diphosphate.

The changes occurring on incubation of the bean starch fraction with radioactive uridine diphosphate glucose are shown in Table 1; these results indicate a correspondence between disappearance of uridine diphosphate glucose, formation of uridine diphospate and incorporation of glucose into the starch fraction.

Table 1. Analysis of Substrates and Products

	μmole	μmole
Disappearance of uridine diphosphate glucose	0·02*	0·016†
Formation of uridine diphosphate	0·017*	0·015‡
Glucose-U-^{14}C incorporated into starch		0·017‡

* Calculated from absorbancy at 260 mμ.
‡ Calculated from radioactivity.
† Estimated with pyruvate kinase (ref. 7*c*).

The identity of the product formed was investigated as follows. The starch fraction after incubation with radioactive uridine diphosphate glucose was thoroughly washed with aqueous ethanol, suspended in water, heated for 10 min. at 100° C. and then treated with β-amylase. Wheat β-amylase was allowed to act for 12 hr. at 37° C., and 3 volumes of methanol were added. The soluble fraction was evaporated, spotted on paper and chromatographed with butanol/pyridine/water (6 : 4 : 3 v/v)[13] as solvent. As shown in Fig. 1*a* the radioactivity was found to migrate like maltose. For further confirmation of the identity of the substance, the 'maltose' eluted from the paper after chromatography was submitted to electrophoresis with borate buffer[14]. As shown in Fig. 1*b*, the radioactivity migrated with the 'maltose'. Under these conditions, cellobiose, laminaribiose and gentiobiose are neatly separated from maltose.

Similar results were obtained with α-amylase. Saliva (0·1 volume) was allowed to act for 30 min. at 37° C. and the samples processed as described for β-amylase. The results were similar except that radioactivity appeared also in the maltotriose spot.

*Post-Doctoral Fellow of the University of Buenos Aires.

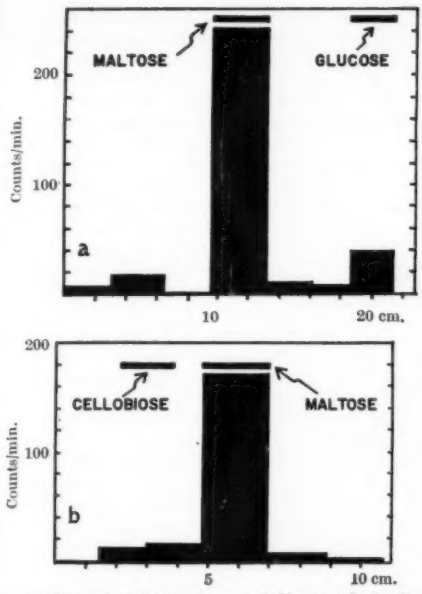


Fig. 1 (*a*) Paper chromatography; and (*b*) paper electrophoresis of reaction product after treatment with β-amylase

These results are consistent with the following formulation:

uridine diphosphate glucose + acceptor → uridine diphosphate + α-glucosyl-1,4-acceptor (1)

in which the acceptor is starch.

Other experiments were carried out in order to study the specificity of uridine diphosphate glucose as glucose donor. As shown in Table 2, glucose phosphates and sucrose showed some incorporation of radioactivity but much less than uridine diphosphate glucose.

Sucrose has been tested as glucose donor in other experiments with various plant tissue preparations with negative results. The well-known ready conversion of sucrose into starch *in vivo* might take place indirectly with uridine diphosphate glucose as an intermediate. The formation of uridine diphosphate glucose from sucrose has been detected (refs. 8, 9) and may be written as follows:

sucrose + uridine diphosphate ⇌ uridine diphosphate glucose + fructose (2)

The addition of reactions (1) and (2) would result in the formation of starch from sucrose.

Table 2. DONOR SPECIFICITY

Bean starch fraction (8 mgm.) incubated 1 hr. under conditions similar to those indicated in Table 1 with about 0·2 mole of substrates indicated

Donor	Counts/min. added	Counts/min. recovered in 'starch' fraction
Uridine diphosphate glucose	5,300	590
Glucose-1-phosphate	8,000	190
Glucose-6-phosphate	10,000	210
Sucrose	8,500	130
Glucose	10,000	0

This investigation was supported in part by a research grant (No. *G*-3442) from the National Institutes of Health, U.S. Public Health Service and by the Consejo Nacional de Investigaciones Cientificas y Técnicas.

[1] Hanes, C. S., *Proc. Roy. Soc.*, B, **128**, 421 (1939).
[2] Ewart, M. H., Siminovitch, D., and Briggs, D. R., *Plant Physiol.*, **29**, 407 (1954).
[3] Rowan, K. S., and Turner, D. H., *Aust. J. Biol. Sci.*, **10**, 414 (1957).
[4] Stocking, C. R., *Amer. J. Bot.*, **39**, 283 (1952).
[5] Sutherland, E. W., *Ann. N.Y. Acad. Sci.*, **54**, 693 (1951).
[6] Larner, J., and Villar-Palasi, C., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1234 (1959). Mommaerts, W. F. H. M., Illingworth, B., Pearson, C. M., Guillory, R. J., and Seraydarian, K., *ibid.*, **45**, 791 (1959). Schmid, R., Robbins, P. W., and Traut, R. R., *ibid.*, **45**, 1236 (1959).
[7] (*a*) Leloir, L. F., and Cardini, C. E., *J. Amer. Chem. Soc.*, **79**, 6340 (1957). (*b*) Leloir, L. F., Olavarría, J. M., Goldemberg, S. H., and Carminatti, H., *Arch. Biochem. Biophys.*, **81**, 508 (1959). (*c*) Leloir, L. F., and Goldemberg, S. H., *J. Biol. Chem.*, **235**, 919 (1960).
[8] Cardini, C. E., Leloir, L. F., and Chiriboga, J., *J. Biol. Chem.*, **214**, 149 (1955).
[9] Leloir, L. F., and Cardini, C. E., *J. Biol. Chem.*, **214**, 157 (1955).
[10] Feingold, D. S., Neufeld, E. F., and Hassid, W. Z., *J. Biol. Chem.*, **233**, 783 (1958).
[11] Aspinall, G. O., and Kessler, G., *Chem. and Indust.*, 1296 (1957), Kessler, G., *Ber. schweiz. bot. Ges.*, **68**, 5 (1958).
[12] Currier, H. B., and Strugger, S., *Protoplasma*, **45**, 552 (1956).
[13] Jeanes, A., Wise, C. S., and Dimler, R. J., *Anal. Chem.*, **23**, 415 (1951).
[14] Consden, R., and Stanier, W. M., *Nature*, **169**, 783 (1952).
[15] Paladini, A. C., and Leloir, L. F., *Biochem. J.*, **51**, 426 (1952).

ANALYSIS OF METABOLIC RATES AT THE CELLULAR LEVEL

By L. G. LAJTHA, R. OLIVER, R. J. BERRY and ELIZABETH HELL

Radiobiology Laboratory, Department of Radiotherapy, Churchill Hospital, Oxford

DURING the course of autoradiographic work with iron-59 and tritium, carbon-14, or sulphur-35 labelled compounds in human bone marrow cells, Ehrlich mouse ascites cells, or in mouse thymus, spleen, and intestine, it was noticed that the grain counts over labelled cells showed a considerable spread. This spread was significantly greater than that for a Poisson distribution about a single mean corresponding to the statistical variation of radioactive disintegrations. In short-term incubations the grain counts over the labelled cells measure uptake during unit time per cell (that is, uptake rate per cell), and some biological variation is natural even in a homogeneous population of cells, so this finding was not unexpected. However, variations in the observed spread warranted a closer analysis of the distributions of grain counts, and these have indicated certain qualitative differences in the uptake pattern of various labelled compounds and in different cell types.

Theoretical Considerations

(1) If the uptake rate is constant throughout the cell population the same amount of radioactive material will be incorporated into each cell, and the variation in the grain count over cells will be entirely due to the statistical fluctuation in the number of disintegrations occurring during the time of exposure. This would lead to a Poisson distribution about a mean value with a standard deviation equal to the square root of the mean. As the number of grains over each cell is necessarily small, this deviation will be appreciable. Thus for a typical mean of 45 grains the standard deviation would be $\sqrt{45}$ or 6·7, that is, about 15 per cent. This distribution is shown in Fig. 1*A*.

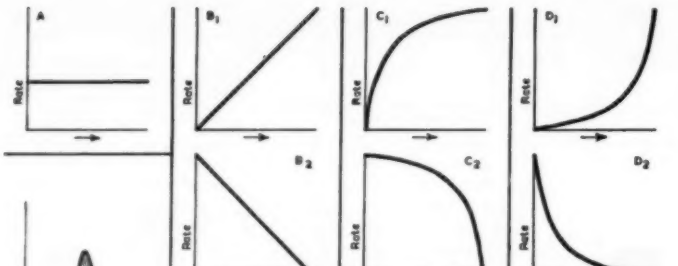


Fig. 1

In a non-growing population of cells a constant rate of uptake may indicate a steady rate of turnover or synthesis of a compound. However, some biological variation would be expected, especially between the young and the senescent members of the population, so that the observed spread would exceed that of the ideal Poisson distribution (Fig. 1*A*) and depend partly on the relative proportions of 'normal' and 'senescent' cells.

In a non-synchronous growing population of cells, theoretically a number of possible variations in the rate of uptake may be considered. A constant rate of uptake would lead to the type of distribution of grain count discussed above.

A linear increase or decrease of the rate of uptake would result in an even distribution of low and high rates and the grain count distribution would ideally follow the symmetrical type of curve shown in Fig. 1*B*. If, however, the rate of uptake increases or decreases exponentially, then the resulting grain count distribution would be asymmetrical as illustrated in Figs. 1*C* and *D*.

(2) In a growing cell population the cells increase their mass during interphase from a '2*n*' to a '4*n*' value to yield two '2*n*' cells after mitosis. Therefore a constant uptake-rate in such a population would imply a synthesis of a compound (or structure) which would not be functional until after mitosis, or a turnover of a compound (or structure) which does not reduplicate until mitosis.

A linear increase in uptake rate would correspond to a constant rate of production of new, for example enzyme, molecules (responsible for the uptake of the compound investigated); however, it would also mean that the 'producer' of the new enzyme molecules is not reduplicated (or if so then is not functional) until mitosis. A linear decrease would imply a similar constant rate of production of an inhibitor of uptake.

Such processes leading to linear changes in uptake rate would seem to be unlikely where the function is continuous throughout the interphase—a condition which would be indicated by effectively all cells of the population being labelled in short-term incubations (100 per cent 'positive' cells). However, where significantly fewer than 100 per cent of the cells are labelled, it can be concluded that the function is active only during part of the interphase. In such cases reduplication can occur in the remaining period, and linear rate changes may be conceivable.

Of the possible forms of exponential type of variations in uptake rate the simplest system would correspond to Fig. 1D_1, where the rate of uptake of a material is proportional to the amount of that material already present in the cell. In such a case a doubling of the rate during interphase would be expected, that is, a 1 : 2 change.

The contrasting situation is represented in Fig. 1D_2, where the rate of uptake of a material is reduced proportionally to the amount of the material in question present in the cell. In such a case a halving of the rate during interphase may be expected.

It is possible to differentiate between these two situations as, although the expected grain count distribution curves would be identical as shown in Fig. 1, the distribution curves for the content of the material in the cell would have the same form as the grain-count distribution curve in case of D_1, but of opposite asymmetry in D_2.

A situation where the uptake rate is limited exponentially, possibly by an exponentially decaying inhibitor, is illustrated in Fig. 1C_1, while an exponential build-up of an inhibitor of uptake rate is represented in Fig. 1C_2. Although the grain-count distribution curves are similar, the content distribution curves would again differentiate between the two situations; for C_1 the asymmetry of the two curves would be similar, for C_2 opposite.

Situations C_1 and C_2 for functions occupying the whole of the interphase (100 per cent positive cell population) would imply birth of an inhibitor during mitosis with subsequent decay in C_1; and build-up of an inhibitor with explosive disappearance during mitosis in the case of C_2. While such situations may exist, neither of them is considered as likely for the majority of cell functions as situations D_1 and D_2. Furthermore, in situations C_1 and C_2 there is no *prima facie* reason why the rate change should be limited to 1 : 2; in fact, rate changes considerably in excess of this could be expected.

In practice, the cell processes may lead to other types of curves (for example, sigmoid or other composite curves), but the above general conclusions on grain-count distribution would still apply. In any event, if the rate changes involved are only of the order of 1 : 2 it may not be possible—as is shown later—to differentiate between linear and exponential or other types of curves. In cases, however, where the rate changes are significantly greater (for situations C_1 and C_2, and also probably in the case of a sigmoid curve), the differences between the various types of curves may become appreciable.

Two special cases, where uptake is related to cell surface area, may be considered. If uptake rate is proportional to total cell surface, then during one duplication of the cell mass uptake rate would increase from 1 : 1·6 only. If, however, uptake rate depends on surface area per unit mass (or mass per unit area) then it would decrease from 1 : 0·8 (or increase from 1 : 1·26) during one duplication of the cell mass. Consequently, if in a growing population of cells a change of uptake rate less than 1 : 2 is observed, this may indicate an

uptake dependent on surface area, but changes from 1:0·8 or 1:1·26 are almost certainly indistinguishable from a constant rate.

Master Curves

The curves shown in Fig. 1 are exaggerated for the sake of illustration of the problem. A master curve was therefore drawn for normal distribution with a standard deviation of 30 per cent, corresponding to standard deviations of 15 per cent for a mean grain count of 45 grains per cell, and of 25 per cent for biological variation. This was likely to represent the greatest spread of grain count distribution which could be expected for a population having a nominally uniform uptake rate.

In order to represent the conditions with a linear or exponential increase or decrease in uptake rate the only case considered was for doubling of the rate during the period of uptake. A composite curve was constructed by the summation of five normal distributions with means at 1·1, 1·3, 1·5, 1·7, and 1·9, and standard deviations of 30 per cent, the relative peak ordinates corresponding to the proportion of cells which would, in each case, lie ideally within that range of uptake rate (1·0–1·2, 1·2–1·4, etc.). This procedure, of course, blurs the clear difference between the types of grain count distribution curve shown in Fig. 1, but was more likely to provide distributions similar to those expected in biological material. The two curves thus calculated for situations *B* and *D* respectively are illustrated in Fig. 2. The asymmetry in the exponential case, although still present, is less marked, and differentiation in practice is unlikely.

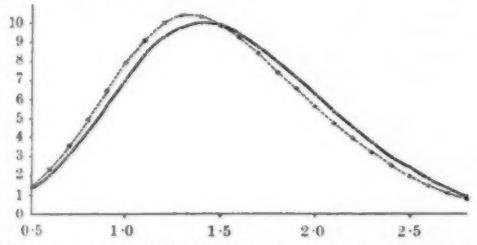


Fig. 2. —, linear *S.D.* 30 per cent; – – –, exponential *S.D.* 30 per cent

In making comparison between these theoretical curves and distributions observed in practice the absolute values of the abscissæ have no significance, only their relative variation. Therefore, to facilitate matching with experimental curves, all the theoretical curves have been re-drawn with linear ordinate scale and a peak value of 10, and with a semilogarithmic abscissa scale along which the curves can be moved without changing their shape.

Experimental Examples

Autoradiographs were prepared from smears of (*a*) human bone marrow cultures after two hours incubation with iron-59, (*b*) human leukæmic lymphocytes after three hours incubation with methionine sulphur-25, and (*c*) mouse thymus imprints 30 min. after administration of thymidine labelled with hydrogen-3. The autoradiographs were exposed long enough to give a mean grain count of 30–50 grains per labelled cell (the optimum grain count depends on the size of the cell and on the background), and grain counts were performed on 300–900 cells of the same type (that is, basophilic normoblasts, small lymphocytes, large thymocytes).

As only a limited number of cells was counted in each experiment the grain count distribution curve obtained was, in general, not smooth. In order to provide a 'best' smoothed distribution the results were considered in ranges of grain count 1–10, 11–20, etc. For each group the total number of cells was plotted against the actual mean value of grain count for that group. For comparison with the theoretical curves the final curve obtained was plotted on semilogarithmic paper with a peak (linear) ordinate of 10. Where more than 500 cells were counted it was still possible to obtain a smooth curve in this way considering ranges in grain count of 5, thus giving a more accurate form of the distribution.

In cases where the proportion of labelled cells in a population is significantly below 100 per cent, it is advisable to over-expose some slides to differentiate between the truly negative and 'false negative' cells. The latter may occur in populations with great ranges in uptake rate.

Caution is indicated with histological sections, as loss of parts of cells may affect the apparent range of uptake rates.

Figs. 3 and 4 show the experimental curve obtained with iron-59 in basophilic normoblasts (dividing population). It is clear that the iron-59 curve does not fit the normal distribution curve with 30 per cent *S.D.* (Fig. 3) but fits the exponential curve (situation *D*) remarkably well (Fig. 4). This, in conjunction with the early appearance of heavily labelled reticulocytes which is observed after a tracer dose of iron-59 (Suit *et al.* (1957); Lajtha and Oliver (1960)) is taken as indication of an exponentially increasing rate of

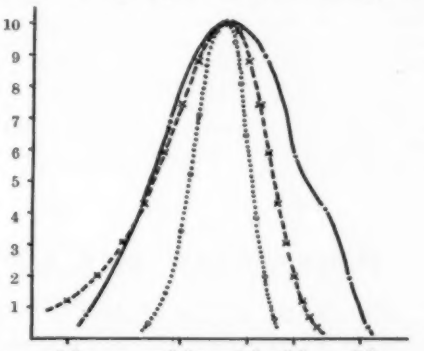


Fig. 3. · · ·, Poisson (mean 45); – – –, normal (*S.D.* 30 per cent); ——, iron-59 experimental

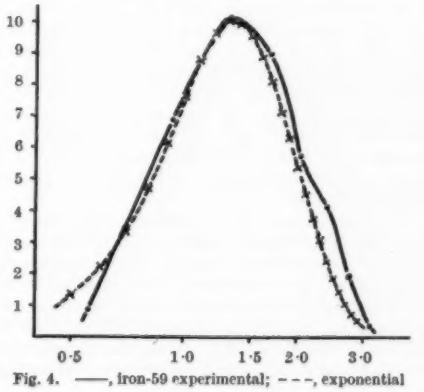


Fig. 4. ——, iron-59 experimental; – – –, exponential

Fig. 5. ·—·, sulphur-35 methionine; ×---×, normal (*S.D.* 30 per cent)

Fig. 6. ——, thymus deoxyribonucleic acid; ---, exponential

uptake of iron-59 (hæmoglobin synthesis) in the dividing population of basophilic normoblasts.

Fig. 5 shows the findings with methionine labelled with sulphur-35 in the small lymphocytes of human leukæmic blood. This experimental curve fits the normal distribution remarkably well, suggesting that the rate of methionine sulphur-35 uptake is constant (? turnover) in this apparently non-growing population of cells (these cells do not synthesize deoxyribonucleic acid).

An entirely different grain-count distribution curve is obtained with thymidine labelled with hydrogen-3 (a deoxyribonucleic acid label) in the large thymocytes (Fig. 6). This curve is considerably broader than the exponential increase from 1 : 2 ; in fact, it indicates a rate difference of the order of 1 : 5. On consideration this finding may not be unexpected ; even if synthesis of deoxyribonucleic acid proceeded at a constant rate along the length of every chromosome, there are significant differences in length between individual chromosomes. In a non-synchronized population of cells with 40 chromosomes (mouse), therefore, cells may occur in which at a given time all 40 chromosomes are in deoxyribonucleic acid synthesis, and others in which, for example, only the last ends of the longest chromosomes. Findings similar to that of Fig. 6 have been obtained with Ehrlich mouse ascites cells (thymidine labelled with hydrogen-3) as well as human bone marrow cells in which formate labelled with carbon-14 was used as deoxyribonucleic acid label (46 chromosomes).

Conclusions

The examples illustrated above indicate that autoradiographic grain-count distribution curves (coupled in some cases with content distribution curves) may be usefully employed in analysis of nature and rates of metabolic processes at the cellular level, information not easily obtained by other methods. Investigations with different labelled precursors in various normal and abnormal cells are in progress ; detailed results will be published elsewhere.

References

Suit, H. D., Lajtha, L. G., Oliver, R., and Ellis, F., *Brit. J. Haemat.*, **3**, 165 (1957).

Lajtha, L. G., and Oliver, R., in "Ciba Symp. Hæmopoiesis" (Churchill, London) (in the press).

TERMINOLOGY IN PLANT- AND SOIL-WATER RELATIONS

By Dr. R. O. SLATYER

Division of Land Research and Regional Survey, Commonwealth Scientific and Industrial Research Organization, Canberra

AND

Prof. S. A. TAYLOR

Department of Agronomy, Utah State University, Logan

At the recent symposium on plant–water relations in arid regions, organized by Unesco at Madrid (*Nature*, **185**, 435 ; 1960), the terminology currently used in this field aroused much interest. The following notes summarize the main points brought forward at a special discussion on this subject. Because of the interest created, the delegates suggested that these notes be published for the information, use and comment of all biological and physical scientists interested.

(1) The water relations of soils and plants have long been described by terms which are thermodynamic in nature, but which have usually been expressed in terms of equivalent pressures. This has led to what can be described as a 'hydraulic' terminology with the terms 'total soil moisture stress' (*TSMS*)[1] and 'diffusion pressure deficit' (*DPD*)[2] being the most common in soil and plant studies respectively. Alternative terms for diffusion pressure deficit, which are commonly used in Europe, include 'suction force'[3] and 'suction pressure'[4]. Alternative terms for *TSMS* include '*pF*' (ref. 5).

(2) While this terminology has frequently provided a satisfactory description of the phenomena observed, its value is limited for the following reasons :

(*a*) The use of pressure terms and units is misleading because in soil and plant systems situations normally exist where a high equivalent pressure or suction can be measured. However, this value represents a potential energy only and no water movement can be expected unless *gradients* of potential energy exist.

(*b*) With reference to the vacuolated plant cell the simple equation :

$$DPD = OP - TP$$

where OP is osmotic pressure and TP turgor pressure, has been in use for many years and has provided a useful interpretation of the cell-water relations of adult mesophytic leaves. Ideally, however, it only refers to a cell which is completely vacuolated. In many plants, the volume of cell wall can reach significant proportions and in xerophytic and sclerophytic tissue the volume of the vacuole can be less than one-half of the total cell volume. Accordingly, rigid use of this simple terminology can introduce errors and may prevent an accurate interpretation and description of the phenomena observed.

(c) Even though the most common terms in the present terminology—'total soil moisture stress' and 'diffusion pressure deficit'—are regarded as pressures and expressed in pressure terms, not only are they thermodynamic in nature but also they are measured by thermodynamic methods—for example, by measuring equilibrium vapour pressure or osmotic potential.

(3) Because of these weaknesses in the present terminology it is thought that a definite step forward would be achieved by adopting more basic thermodynamic terms and units which would enable all the factors contributing to the thermodynamic state of water to be adequately described and assessed. Both diffusion pressure deficit and total soil moisture stress are conceptually expressions of the Gibbs free energy of the water in plant and soil systems respectively. To this extent the reversion to thermodynamic terms and units is a simplification rather than a complication. Several authors, notably, Edlefsen[6], Edlefsen and Anderson[7], Broyer[8–10] and Taylor[11], have already suggested appropriate thermodynamic terminologies.

(4) The free energy function is considered to be the most appropriate thermodynamic function and the most appropriate term is considered to be 'water potential', that is, the chemical potential of the water in the plant or soil. Thus, water potential is the difference between the partial specific Gibbs free energy of water in the system under consideration and of free pure water at the same temperature (which is the reference state). There are several alternative methods for expressing water potential, as can be seen from the thermodynamic identity :

$$\Delta\mu_w = R_w T \ln a_w = R_w T \ln p/p_0 = V_w(P - P_0)$$

where $\Delta\mu_w$ is the water potential, R_w the gas constant per gram of water, T the absolute temperature, a_w the activity of water, p/p_0 the relative vapour pressure, V_w the specific volume of water and $(P - P_0)$ the pressure difference that can be associated with, for example, hydraulic head or turgor pressure.

(5) If we choose pressure (P) and mass fractions of water (n_w) and of solutes (n_j) as independent variables, the differential form of the Gibbs equation for the component water (μ_w) can be written for the homogeneous isothermal state as follows :

$$\mathrm{d}\mu_w = \left[\frac{\partial\mu_w}{\partial P}\right]_{n_w,n_j} \mathrm{d}P + \Sigma_j \left[\frac{\partial\mu_w}{\partial n_j}\right]_{P,n_w,(n_j-1)} \mathrm{d}n_j + \left[\frac{\partial\mu_w}{\partial n_w}\right]_{P,n_j} \mathrm{d}n_w$$

where the subscripts outside the brackets indicate the variables that are held constant in the evaluation. The component $\left[\frac{\partial\mu_w}{\partial P}\right]_{n_w,n_j}$ is the specific or molar volume of water (V_w), and is found by plotting the change of μ_w with respect to changes in P for the water in the soil and plant system. It can be identified as the turgor pressure in plants or as hydraulic pressure in saturated soils. The component

$$\left[\frac{\partial\mu_w}{\partial n_j}\right]_{P,n_w,(n_j-1)}$$

is the slope of the curve found by plotting water potential as a function of the concentration of one solute, with pressure, water content and all the other solute concentrations held constant. It is summed over all the j components to give a complete osmotic potential. Following the notation of Guggenheim (ref. 12, p. 207) the term is given the symbol $D\mu_w \left[= \Sigma_j \left(\frac{\partial\mu_w}{\partial n_j}\right)_{P,n_w} \mathrm{d}n_j\right]$. This term is analogous to that of 'osmotic pressure' now in use. The term $\left[\frac{\partial\mu_w}{\partial n_w}\right]_{P,n_j}$ is the slope of the curve found by plotting the water potential μ_w against water concentration at constant pressure and solute concentration. This is the moisture retention curve and is associated with the soil moisture tension or soil suction as now used. The term will also describe the condition of water within the cell walls or the colloidal system of the protoplasm which cannot be accounted for by turgor pressure or osmotic potential. It is effectively measured as a 'water-release' relationship for plant tissue.

(6) As written, the above expression refers only to isothermal situations. If required, an entropy term can be introduced to permit description of non-isothermal situations :

$$\mathrm{d}\mu_w = \left[\frac{\partial\mu_w}{\partial P}\right]_{T,n_w,n_j} \mathrm{d}P + \Sigma_j \left[\frac{\partial\mu_w}{\partial n_j}\right]_{P,T,n_w,(n_j-1)} \mathrm{d}n_j + \left[\frac{\partial\mu_w}{\partial n_w}\right]_{P,T,n_j} \mathrm{d}n_w + \left[\frac{\partial\mu_w}{\partial T}\right]_{P,n_w,n_j} \mathrm{d}T$$

where T is temperature and $\left[\frac{\partial\mu_w}{\partial T}\right]_{P,n_w,n_j} = -S_w$, where S_w is the specific or molar entropy of the soil or plant water.

(7) Although the differential form of these expressions is more comprehensive and descriptive, the integral form is more straightforward and provides a more direct transition from the existing terminology. For example, one can write :

$$\Delta\mu_w = V_w \Delta P + D_\pi\mu_w + \int_1^{n_w} \tau.\mathrm{d}n_w$$

when $\Delta\mu_w$ is the water potential, V_w is the specific volume of water, ΔP is turgor pressure or hydrostatic pressure, $D_\pi\mu_w = \int_0^{n_j} D\mu_w$ is the osmotic potential, and $\int_1^{n_w} \tau.\mathrm{d}n_w$ is the matric (adjectival form of 'matrix') potential, τ is the slope of the water-release curve and n_w the water content expressed as a mass fraction, but which can be expressed in any concentration unit that is consistent with the units used for expressing τ.

Normally $\Delta\mu_w$ will be negative since the potential of soil or plant water is less than that of free pure

water which is the reference. In plant and soil systems ΔP will normally be positive since turgor pressure and hydraulic pressure are usually greater than atmospheric pressure. In applications to unsaturated soil ΔP can be either positive as in the pressure membrane apparatus or negative as with water inside a tensiometer. The osmotic potential ($D_\pi\mu_w$) will be negative or zero since the concentration of solutes in soil or plant solutions is never less than in free pure water. The water content (n_w) will always be less than pure water since the soil or plant will dilute the water and the complete matric potential will be negative.

As systems become more complex and more independent variables are necessary to describe the status of water in the system; additional terms may be added to the thermodynamic equation to include the new variables. Likewise, external force fields such as those of gravity and electricity can be accounted for by adding terms to the equation (ref. 12, pp. 403, 410).

(8) While these expressions may appear more complicated than the more straightforward hydraulic equations, it is apparent that the same components can be identified and the same techniques can be employed in measurement. For example, the water potential ($\Delta\mu_w$) may be measured in plant tissues using the same methods as are at present employed for measuring DPD [13–15]. It may be measured in soil by determining the equilibrium vapour pressure of soil cores[16–18] or by measuring the several components separately and adding them together. The osmotic potential ($D_\pi\mu_w$) will be measured in plant systems in the same manner as the osmotic pressure of the cell sap is at present measured[13]. Likewise, it may be measured in soil systems by determining the osmotic potential of the extract at saturation and adjusting the value to actual soil water contents[16]. The hydrostatic or turgor pressure (ΔP) is not easily measured in plant tissues. In soils it is measured with piezometers. The slope of the water-release curve (τ) can be obtained by simultaneous measurements of $\Delta\mu_w$ and water content. This may be effectively obtained for soils by the use of pressure membranes, pressure plates or tensiometers that are permeable to solutes. Vapour pressure equipment[17,18] may also be used, providing adjustments are made for the solutes that are present. Weatherley and Slatyer[19] have described an effective method for plants.

(9) At present the most common units of expression are the pressure units of atmospheres or bars. In order to interpret these values in free energy units, it is desirable that a unit of similar numerical size be adopted. The unit of joules kgm.$^{-1}$ is suggested, although ergs gm.$^{-1}$ could be used without difficulty. One erg gm.$^{-1}$ is equivalent to a pressure of one dyne cm.$^{-2}$ multiplied by the specific volume of water (which is unity). The numerical conversion from units of pressure to units of energy may be made as follows:

1 atm. = 1·013 bars = 1·013 × 10^6 dynes cm.$^{-2}$ = 1033·2 cm. water
1 joule kgm.$^{-1}$ = 10^4 ergs gm.$^{-1}$ ≒ 10^4 dynes cm.$^{-2}$ = 10^{-2} bars = 1 centibar
1 bar = 10^6 dynes cm.$^{-2}$ ≒ 100 joule kgm.$^{-1}$ = 10^6 ergs gm.$^{-1}$ = 1019·7 cm. water

1 Richards, L. A., and Wadleigh, C. H., in "Soil Physical Conditions and Plant Growth", ed. Shaw, B. T., 73 (Academic Press, Inc., New York, N.Y., 1952).
2 Meyer, B. S., *Plant Physiol.*, **20**, 142 (1945).
3 Ursprung, A., and Blum, G., *Ber. deut. bot. Ges.*, **34**, 88 (1916).
4 Stiles, W., *Biochem. J.*, **16**, 727 (1922).
5 Schofield, R. K., Trans. Third Int. Cong. Soil Sci., **2**, 37 (1935).
6 Edlefsen, N. E., Amer. Geophys. Union Trans. of 1941, Part 3, 22, 917 (1941).
7 Edlefsen, N. E., and Anderson, A. B. C., *Hilgardia*, **15**, 31 (1943).
8 Broyer, T. C., *Bot. Rev.*, **13**, 1 (1947).
9 Broyer, T. C., *Plant Physiol.*, **26**, 589 (1951).
10 Broyer, T. C., *Plant Physiol.*, **26**, 655 (1951).
11 Taylor, S. A., *Soil Sci.*, **86**, 83 (1958).
12 Guggenheim, E. A., "Thermodynamics", third ed. (North Holland Publishing Co., Amsterdam, 1957).
13 Crafts, A. S., Currier, H. B., and Stocking, C. R., "Water in the Physiology of Plants" (The Ronald Press Co., N.Y., 1949).
14 Spanner, D. C., *J. Exp. Bot.*, **2**, 145 (1951).
15 Slatyer, R. O., *Aust. J. Biol. Sci.*, **11**, 349 (1958).
16 Richards, L. A., "Diagnosis and Improvement of Saline and Alkali Soils", U.S. Dept. Agric. "Agric. Handbook", No. 60 (1954).
17 Richards, L. A., and Ogata, Gen, *Science*, **128**, 1089 (1958).
18 Monteith, J. L., and Owen, P. C., *J. Sci. Instr.*, **35**, 443 (1958).
19 Weatherley, P. E., and Slatyer, R. O., *Nature*, **179**, 1085 (1957).

A GENETICALLY DETERMINED ENZYME IN THE PIG

By Dr. K.-B. AUGUSTINSSON and Dr. B. OLSSON

Institute of Organic Chemistry and Biochemistry, University of Stockholm, and Department of Animal Nutrition, Genetics and Hygiene, Royal Veterinary College, Stockholm

PIG blood plasma contains two esterases, an acetylarylesterase and a butyrylcholinesterase[1,2]. Both enzymes hydrolyse aromatic esters, for example, phenyl acetate, arylesterase being approximately 100 times more active toward this substrate than cholinesterase when hydrolysis rates are compared on a nitrogen content basis. In contrast to the activity of cholinesterase, the arylesterase activity exhibits considerable individual variation[3] with activity values ranging from 0 to 200. In previous reports[2,3] the values for arylesterase activity were expressed per 0·1 ml. plasma (instead of 0·02 ml., as in this communication) and were not corrected for cholinesterase activity. These values, therefore, are not directly comparable with those given above; they may be recalculated, however, by dividing previous values by 4 and subtracting with 10, which is the mean activity value per 0·02 ml. plasma for cholinesterase hydrolysing phenyl acetate.) These values were obtained with whole plasma and phenyl acetate in the presence of prostigmine, which selectively inhibits cholinesterase. Plasmas from all new-born piglets so far studied had very low activity in hydrolysing phenyl acetate. We have recently demonstrated[3] that the low activity at birth and also of certain adult animals is almost entirely due to cholinesterase, and is not due to the presence of an inhibitor. Previous experiments also demonstrated that the increase in plasma arylesterase activity of some piglets during the first weeks of life runs parallel with the increase in plasma albumin concentration, and that this increase in activity is gene-controlled. It was not possible, however, to explain how the various activity-levels are genetically

determined and/or which type of gene interaction is most likely.

The results obtained recently in work with a great number of cross tests, including back-crosses, with pigs of various phenotypes have confirmed our proposal that the synthesis of plasma arylesterase is genetically controlled and made it possible to put forward the following hypothesis for gene interaction in the formation of this enzyme. The various phenotypes of adult animals are produced by the interaction of multiple allelic genes :

Allelic gene	Activity-level
a	0
A_1	25
A_3	50
A_2	75
A_4	100

Each allelic gene determines a certain plasma arylesterase activity and the distinguishable phenotypes among adult animals (at least two months of age) are produced as the manifestation of additive effects of two allelic genes, for example :

Genotype	Phenotype
aa	0
aA_3	50
A_1A_1	50
A_1A_3	100
A_3A_4	175

This hypothesis is strongly supported by the experimental results obtained, as will be briefly reported in the following. A full report will be published in *Hereditas*.

Matings were performed with animals of both Swedish Landrace (*Svensk lantras*) and Yorkshire breeds. No differences in gene interaction for the synthesis of plasma arylesterase have been observed between these breeds, pure or mixed. There is no sex influence upon this synthesis.

In our first experiments the phenotypes could be divided into three groups, corresponding to the esterase activities 75, 25 and 0. One sow from each group of the offspring obtained after crossing a boar of phenotype 0 (zero) with sows of various phenotypes was used in back-cross tests. A sow of phenotype 100 segregated into two phenotypes, 75 and 25. In back-cross tests, sows of either of these phenotypes again segregated into two groups, 75 and 0, and 25 and 0, respectively. It was concluded that the father was homozygous for the gene a responsible for 0 (zero) activity and that two allelic genes, A_3 and A_1, determined the activity of phenotypes 75 and 25, which consequently corresponded to the heterozygous genotypes aA_3 and aA_1. Similar results were obtained in matings with sows of phenotype 50 (genotype aA_3) and the same father. These experiments supported the hypothesis that four allelic genes produced the various phenotypes.

In order to obtain further evidence for the hypothesis, the progeny of a great variety of types of mating was studied. In all matings where both parents were of phenotype 0, the offspring were always of this same phenotype. When two sows of the same phenotype 100 were crossed with the same boar of phenotype 0 (genotype aa), the offspring in the two matings differed. There were two phenotypes (75 and 25) in one litter and only one (50) in the other. This means that the first sow was heterozygous for the genes A_3 and A_1, and the other sow homozygous for A_2. When both parents had the same plasma arylesterase activity (75), three phenotypes were obtained in the offspring in accordance with Mendelian inheritance. It was easily concluded that the genotype of both parents was aA_3 and the three phenotypes of the offspring corresponded to three kinds of genotypes, A_3A_3, aA_3 and aa. The offspring of a boar of phenotype 25 crossed with a sow of phenotype 125 segregated into three phenotypes, one of which (75) could correspond to two genotypes, aA_3 or A_1A_2. The existence of a series of multiple allelic genes was also supported by the observation of four phenotypes in the progeny of mating a boar of phenotype 125 and a sow of phenotype 100, the genotypes of which were A_2A_3 and A_1A_2, respectively. A similar segregation was obtained in the following mating :

♀ 75 aA_3 × ♂ 25 aA_1

1♀ 2♂	1♂	2♀ 2♂	2♀ 1♂
0	25	75	100
aa	aA_1	aA_3	A_1A_3

In the series of mating performed, three various genotypes, A_1A_3, A_2A_3 and aA_4, were observed producing the same phenotype 100. These and a number of similar results strongly support the hypothesis that the biosynthesis of plasma arylesterase in adult pigs is genetically determined, and that the activity of this enzyme is the manifestation of additive effects of allelic genes.

The biochemical reactions determining this genetically controlled esterase activity are unknown. It has been demonstrated that the variation in activity is not due to the presence of activators or inhibitors. The various activities observed may be due to the same enzyme protein, the concentration of which varies or the specific activity of which is different in various phenotypes. The first explanation is the more likely, since it would be difficult otherwise to explain the rise in activity for certain groups in a litter during the course of lactation and also the various activity-levels observed. A third possibility is that the various activity-levels are due to the presence of different enzyme molecules of various turnover numbers, but this explanation seems unlikely for the same reasons. No differences in properties of the activity of various phenotypes have been observed. We therefore conclude that the various activity-levels found experimentally actually reflect various concentrations of the same enzyme protein, the synthesis of which is genetically determined by a series of multiple allelic genes. This synthesis is probably enzymatically controlled, and the activity of the enzyme(s) involved is (are) directly influenced by gene action.

We have no explanation so far as to the mechanism underlying the formation of the series of allelic genes. The existence of multiple activity-levels makes likely the occurrence of duplicate, triplicate and quadruplicate genes, corresponding to the allelic genes A_2, A_3 and A_4. This means that these alleles are to be regarded as the result of duplication, etc., of gene A_1 at the same locus in the chromosome.

This investigation has been supported by grants from the Swedish Medical Research Council. We are grateful to Prof. A. Hansson and Dr. S. Skårman, Animal Breeding Institute, Wiad, Sweden, for permission to use the livestock of that Institute.

[1] Augustinsson, K.-B., *Nature*, **181**, 1786 (1958); *Acta Chem. Scand.*, **13**, 571 (1959).
[2] Augustinsson, K.-B., and Olsson, B., *Biochem. J.*, **71**, 477 (1959).
[3] Augustinsson, K.-B., and Olsson, B., *Biochem. J.*, **71**, 484 (1959).

LETTERS TO THE EDITORS

ASTROPHYSICS

The Solar Flare of June 1, 1960

A SOLAR flare in the 3+ category of importance and of exceptionally large extension was observed on June 1 at the Royal Greenwich Observatory, Herstmonceux, and at a number of other solar stations in Europe. We have recently been analysing the film in $H\alpha$ light taken with the Lyot heliograph at the Royal Observatory, Cape of Good Hope, which gives a complete record of the flare at 1-min. intervals under cloudless conditions.

The spot group over which the flare occurred was of complex E-type containing 24 spots and had extended rapidly in area since its first appearance on the east limb on May 29. The flare began at 0823 U.T. and developed rather slowly until about 0837 when the 'flash' phase occurred with a rapid increase in area and intensity at the main centre (N., 30° lat.; E., 50° long.). The greatest extent of the emission filaments was recorded at about 0900 (Fig. 1) when the measured area was 27 (unit = 1 sq. degree heliographic at the centre of the solar disk). If we correct for foreshortening, the heliocentric angle (h) being 54°, we obtain a corrected area of 46 square degrees, or 2,230 millionths of the hemisphere, which makes this flare one of the largest observed in recent years and comparable in area with that of July 25, 1946.

From the measured co-ordinates we have replotted the shape of the flare as it would have appeared had it been seen on the Sun's central meridian. The emission filaments in this way can be seen to extend across the spot group in a north-east – south-west direction for a distance of 290,000 km., and some of the isolated outlying bright regions were located 200,000 km. to the south of the main centre.

Fig. 1. Class 3+ flare of June 1, 1960 (0900 U.T.), recorded with the Lyot $H\alpha$ heliograph at the Royal Observatory, Cape of Good Hope

The Tatsfield Receiving Station of the B.B.C. (engineer-in-charge, H. V. Griffiths) reported a very severe sudden ionospheric disturbance occurring from 0837 until 1200, absorption remaining greater than normal until about 1400. In the peak period about 0900 all communications at high frequency were affected.

In the geomagnetic field a sudden commencement was recorded at 1800 on June 3, and this was followed by a minor storm (K_p max. = 7) lasting for two days. However, the distance of the flare from the Sun's central meridian reduced the probability that a magnetic storm would follow, and the long time interval between the flare and the sudden commencement, s.c. (57 hr.), suggests that this particular storm was caused by particles emitted from a subsequent flare.

A fuller account of the flare will be published elsewhere with accompanying light-curves. We wish to express our thanks to the staff at the Royal Observatory, Cape of Good Hope (under Prof. R. H. Stoy), for the fine film record; J. Churms, in charge of the Lyot heliograph, assisted by Mrs. E. Fiamingo, J. D. Laing, R. Lake and D. S. Malan.

M. A. ELLISON
SUSAN M. P. MCKENNA
J. H. REID
Dublin Institute for Advanced Studies,
School of Cosmic Physics,
Dunsink Observatory.

Effect of Upper Atmosphere Wind on the Rotation of Satellite 1958δ1

CHANGES in the rotation of 1958δ1 (*Sputnik* 3 rocket) can apparently be explained by, and give additional evidence of, strong westerly wind in the upper atmosphere. According to Merson, King-Hele and Plimmer[1] such air-flow, accompanying the Earth's rotation, accounts for observed effects in the movement of the orbital planes of satellites.

The rotation period of 1958δ1, estimated from its regular fluctuations of brightness, increased at a decreasing rate, from just over 8 sec. in June 1958, to about 9·5 sec. in mid-November. It then decreased by at least 0·3 sec. during the last few days of flight, as noted, for example, by Carr, Stewart and Senne[2]. Late in July 1958, the rate of increase decreased significantly. This effect is mentioned by Esipova and Zverev[3]. It coincided with entry of the perigee into denser tropical atmosphere; King-Hele and Walker[4] found a change in the rate of decay of the orbital period at this point.

Air resistance at perigee alone would be expected to exert a progressively increasing deceleration of spin. The observed behaviour reveals an opposing acceleration, which increased with the air density at perigee, and in the end became dominant.

Esipova and Zverev determined from visual observations that the rotation axis was transverse

to the rocket axis, giving end-over-end tumbling. Since the observed 'flash period' always increased slightly with increasing flight range of observation, the rotation was retrograde with respect to the west-to-east transits observed and, therefore, clockwise as viewed from the north.

Such rotation would be most sensitive to accelerating 'windmill' behaviour, generated by the cross-wind, when the satellite passed northwards through perigee in the relatively dense and fast circulating tropical atmosphere. This condition would apply from late July 1958 onwards, and the effect is likely to have increased considerably when the altitude decreased shortly before fall-in.

Each feature of the rotation thus appears to be accounted for, perhaps more convincingly than in terms of meteorite impact, material ablation or even forces of electrical or magnetic origin. It may be that only very long, light-weight objects such as cylindrical balloons and empty rocket cases will show this type of response.

C. R. Faulkner

7 Womma Road,
Elizabeth North,
South Australia.

[1] Merson, R. H., King-Hele, D. G., and Plimmer, R. N. A., *Nature*, **183**, 239 (1959).
[2] Carr, P. H., Stewart, R. M., and Senne, J. H., *Amer. J. Phys.*, **28** (1), 64 (1960).
[3] Esipova, M. I., and Zverev, M. S., *Astr. Sovet an SSSR.*, B, **4**, 12 (1959). (R.A.E. Farnborough Lib. Trans. 842.)
[4] King-Hele, D. G., and Walker, D. M. C., *Nature*, **182**, 860 (1958).

RADIOPHYSICS

Possibility of detecting Ionospheric Drifts from the Occurrence of Spread *F* Echoes at Low Latitudes

During the course of an investigation concerning the geographical extent of the scattered and diffuse echoes frequently observed during the night hours at high and low magnetic latitudes (spread *F*) an interesting phenomenon was observed. Times of occurrence of spread *F* echoes on ionospheric soundings taken every 15 min. during September and October 1957 at four stations in Peru (Talara, Chiclayo, Chimbote and Huancayo—average separation about 350 km., magnetic dip from 2° to 13°) strongly suggested that the patches of irregularities thought to be producing the spread *F* were often observed successively at each of the four stations. For example, an occurrence of spread *F* might be observed to begin at, say, 0015 hr. at Talara, the north-westernmost station, at 0045 at Chiclayo, at 0100 at Chimbote and at 0145 at Huancayo, the south-easternmost station. This tendency was particularly marked for the relatively short occurrences (less than about 2 hr. in duration) that were commonly observed during the latter half of the night. As the stations were located approximately along a straight line, it is not possible to deduce unambiguously the magnitude and direction of the suggested drift. It has been shown, however, that the geographic north–south component of the night *F* region drift is usually small at these latitudes[1]. Therefore, on the assumption that the drift was wholly in the magnetic east–west direction, magnitudes of the geographic east–west component of the apparent drifts indicated by the spread *F* occurrences during September and October have been estimated. A mean apparent drift velocity of 135 m./s. towards the east was obtained for period 0000–0400 hr. Skinner, Hope and Wright, using the Mitra technique at another equatorial location (Ibadan) during the same period, observed a mean east–west component of *F* region drift of about 110 m./s. towards the east[2]. The degree of agreement suggests that drifts, either of the patches of irregularities giving rise to the spread *F* echoes or of the disturbance producing the irregularities, may play an important part in the occurrence of spread *F* at low latitudes.

A more comprehensive study of spread *F* occurrences along the Peruvian chain of stations during the International Geophysical Year period, including a full interstation correlation analysis, is now being completed and will be published elsewhere.

R. W. Knecht*

National Bureau of Standards,
Boulder, Colorado.
Aug. 3.

* On leave at the Cavendish Laboratory, Cambridge, England.

[1] Purslow, B. W., *Nature*, **181**, 35 (1958).
[2] Skinner, N. J., Hope, J., and Wright, R. W., *Nature*, **182**, 1363 (1958).

PHYSICS

Thermal Expansion at Low Temperatures

Considerable experimental data have been published[1] on the heat capacity C of solids, extending to temperatures which are very low in relation to their characteristic temperature θ; data are available in the temperature region $T \sim \theta/100$, where the Debye continuum model may be expected to apply and where for metals the electronic contribution is important. Complementary information regarding the thermal expansion coefficient, α, is desirable as it would throw additional light on the lattice dynamics of solids, particularly the anharmonic nature[2] of the interatomic forces as evidenced by the Grüneisen parameter $\gamma = 3\alpha\chi V/C$ (χ = incompressibility, V = volume). Also any data obtained about an electronic expansion coefficient, α_e, in metals should provide useful information about their band structures.

Unfortunately, most methods of measuring the expansion, whether by X-rays[3], interferometry[4] or capacitance[5], have not been sufficiently sensitive to trace its variation much below $\theta/10$. However, there are now available techniques for the accurate comparison of small capacitances which allow movements as small as 10^{-9} cm. to be detected, and these may be used to determine expansion coefficients down to $T \sim \theta/100$. Such techniques depend on the use of three-terminal capacitors in which the capacity to be measured is a mechanically well-defined gap between two of the terminals (or conducting surfaces) and the third terminal is a conductor which acts both as a guard ring and as a shield surrounding the other two. This unknown capacitance is then compared with a reference capacitance in a four-arm bridge of which the two other arms are tightly coupled inductive arms; ground-lead capacitances do not appear in the balance equations but merely affect the bridge sensitivity. Some recent developments in precise capacitance measurement have been described by Thompson[6] and their application to measuring thermal expansion will be described elsewhere[7]. The measurements described here on the thermal

expansion of copper and iron were made with a capacitance-bridge kindly loaned by Mr. A. M. Thompson, of the Commonwealth Scientific and Industrial Research Organization Division of Electrotechnology.

An expansion 'cell' was made from O.F.H.C. copper (plus brass screws and mica insulating washers) which is a cylinder about 6 cm. long and 5 cm. diameter; in it is mounted a bobbin-shaped specimen of 5-cm. length with parallel lapped faces of 2-cm. diameter at each end. One end is fixed and the other (free) end-face forms a parallel-plate condenser with a 1·3-cm. diameter copper plate distant about 0·02 cm. Variations in the relative length of specimen and copper cell alter this 0·02-cm. gap and hence alter its capacitance. With our present bridge sensitivity of 10^{-6} pF, the 5 pF capacitance formed by this gap may be measured to 1 in 5×10^6, corresponding to $0{\cdot}5 \times 10^{-8}$ cm.; this amounts to about 1 part in 10^9 for a system of length 5 cm.

The expansion cell is mounted in a conventional cryostat[8] so that its temperature may be varied from 1·2° to 300° K. Thus far, most measurements have been confined to the region below 25° K. on specimens of (*A*) annealed O.F.H.C. copper, (*B*) sintered beryllium, kindly supplied by the Australian Atomic Energy Commission through Mr. G. L. Hanna, of its Metallurgy Section, (*C*) annealed 99·96 per cent iron, kindly supplied by the British Iron and Steel Research Association. Hence data have been obtained on the relative changes in length of (*A*) copper versus copper, (*B*) copper versus beryllium, (*C*) copper versus iron.

For system (*A*) the variation of gap with temperature is very small, confirming that the cell is mechanically stable.

For system (*B*) the relative measurements on copper–beryllium give values of the expansivity of copper if α(beryllium) $\ll \alpha$(copper). At low temperatures ($T < \theta/10$) we expect this to be true, at least for the lattice vibrational contribution, α_g, since to a first approximation:

$$\alpha_g(\text{beryllium})/\alpha_g(\text{copper}) \simeq C_g(\text{beryllium})/C_g(\text{copper}) \simeq [\theta(\text{copper})/\theta(\text{beryllium})]^3$$

and θ(copper) is more than three times smaller than θ(beryllium); here C_g is the lattice component of the specific heat. It appears partially confirmed by our data which then yields $\alpha = 0{\cdot}59_3 \times 10^{-6}$ per deg. C. as compared with a value $\alpha = (0{\cdot}58 \pm 0{\cdot}04) \times 10^{-6}$ per deg. C. obtained by Rubin *et al.*[4] at 25° K., their lowest temperature. However, this does not preclude beryllium from exhibiting a small electronic coefficient α_e, proportional to temperature (rather than T^3 as for α_g), which may be important in the evaluation of our results below 5 or 10° K. Concerning α_e, Visvanathan[9] has deduced theoretically that the electron gas should be responsible for a thermal expansion coefficient given by $3\alpha_e = aC_e/\chi V$, where C_e is the electronic heat capacity. The numerical constant a (sometimes denoted γ_e) should be 2/3 for a free electron gas, and for copper this leads to $\alpha_e \simeq 1{\cdot}5 \times 10^{-10} T$ per deg. C.; for beryllium it would lead to about half this value for α_e if the assumption $a = 2/3$ were true.

Assuming the expansivity of beryllium to be negligible, the data on system (*B*) give the following results for copper:

(1) No evidence of change in length greater than about 1 Å. could be detected between 1 and 4° K. If we assume that $\alpha = \alpha_e + \alpha_g = AT + BT^3$, then this limits the term $\alpha_e = AT$ to be not greater than $2 \times 10^{-10} T$ and the second term to $3 \times 10^{-11} T^3$. Theoretically α_e should be slightly less than this limiting value and may be partially masked by a similar term in beryllium.

(2) Analysis of the changes in length observed in the region from 4 to about 9 or 10° K. indicated that $\alpha \simeq (3{\cdot}0 \pm 0{\cdot}3) \times 10^{-11} T^3$ per deg. C.

(3) Two sets of values for the Grüneisen parameter were calculated as follows: a value γ_T(total) = $3\alpha\chi V/C$, where $C = C_e + C_g$ and a value γ_g(lattice) = $3\alpha_g\chi V/C_g$. Values of γ_T were sensibly constant at $1{\cdot}85 \pm 0{\cdot}05$ down to about 12° K. but fell rapidly below that, reflecting the apparent absence of any linear term in α to counterbalance the linear term in C. However, γ_g remains fairly constant to the lowest temperatures. Below 10° K., values $\gamma_g = 1{\cdot}9 \pm 0{\cdot}2$ follow from the relation $\alpha = (3{\cdot}0 \pm 0{\cdot}3) \times 10^{-11} T^3 = \alpha_g$ and the established data that $C_g = 1946\ (T/345)^3 j/g$ mole deg.

For the system (*C*) of iron versus copper, changes in length were observable down to 1·5° K. These were approximately proportional to T^2 at the lowest temperatures, corresponding to an expansion coefficient varying as T. Above 4° K. they were corrected for the expansion of copper using data obtained on system (*B*). Results may be summarized for iron as follows:

(1) Below 8° K., $\alpha \simeq (0{\cdot}26 \pm 0{\cdot}04) \times 10^{-8} T$ per deg. C.; assuming that this linear term is the electronic coefficient α_e, then we find that $a = \gamma_e = \alpha_e\chi V/C_e \simeq 1{\cdot}7$.

(2) Between 8° and 15° K., results can be represented by $\alpha = 0{\cdot}26 \times 10^{-8} T + (1{\cdot}05 \pm 0{\cdot}1) \times 10^{-11} T^3$; if the second term is identified with α_g, then $\gamma_g \simeq 2{\cdot}0 \pm 0{\cdot}2$. Much of the uncertainty in the expansion coefficient above 8° K. arises because the correction for copper is substantial.

(3) Data obtained in the region 55–90° K. give values of γ_T ranging from 1·62 to 1·68 and measurements between 20 and 15° K. give $\gamma_T = 1{\cdot}8 \pm 0{\cdot}1$. These may be compared with a room temperature value of about 1·7.

Apart from taking further measurements on the system (*C*) between 10° and 50° K., it is hoped to: (*a*) obtain absolute values of α for copper using a different form of expansion 'cell' in which the specimen may be raised in temperature while the 'cell' is fixed at the temperature of a liquid bath. Such a cell has been completed and seems to work satisfactorily; (*b*) data for sapphire relative to copper in the existing 'cell' should also yield α values for copper since α for sapphire should be negligible at the lowest temperatures; (*c*) data on the electronic expansion coefficient for chromium and for palladium, (*d*) data for lead relative to copper between 1° and 10° K. where we should expect α_g(lead) $\gg \alpha_g$(copper), and (*e*) data on the electronic expansion coefficient for the superconductors aluminium and niobium would be of great interest. Earlier measurements[10] of the change in dimensions of aluminium when superconductivity is destroyed by a magnetic field suggest that α_e should be surprisingly large; for niobium the large electronic heat capacity in the normal state and its high transition temperature make it an interesting study.

In conclusion, it appears from these measurements that iron exhibits a component of thermal expansion arising from the free electrons. In copper no such component has been found as yet although values

for the Grüneisen parameter have been established down to below 10° K.

I am most grateful to my colleagues Messrs. A. F. A. Harper, W. R. G. Kemp and A. M. Thompson for their interest and advice.

GUY K. WHITE

Division of Physics,
Commonwealth Scientific and
Industrial Research Organization,
Sydney.
July 14.

[1] Review by Keesom, P. H., and Pearlman, N., "Handb. der Physik", **14**, 282 (1956).
[2] Barron, T. H. K., *Phil. Mag.*, **46**, 720 (1955).
[3] Simmons, R. O., and Balluffi, R. W., *Phys. Rev.* **108**, 278 (1957).
[4] Rubin, T., Altman, H. W., and Johnston, H. L., *J. Amer. Chem. Soc.*, **76**, 5289 (1954).
[5] Bijl, D., and Pullan, H., *Physica*, **21**, 285 (1955).
[6] Thompson, A. M., *Inst. Rad. Eng. Trans. Instrumentation*, **I-7**, 245 (1958).
[7] White, G. K., to be submitted to *Cryogenics* (Heywood, London).
[8] White, G. K., "Experimental Techniques in Low-Temperature Physics", 158 (Oxford University Press, 1959).
[9] Visvanathan, S., *Phys. Rev.*, **81**, 626 (1951).
[10] Olsen, J. L., and Rohrer, H., *Helv. Phys. Acta*, **30**, 49 (1957).

Effect of Temperature on the Expansion on Setting of Gypsum Plaster

IT has been shown by Ridge[1] that setting-temperature exerts a profound effect on the structure of set gypsum plaster. He postulated that this change in structure would be accompanied by changes in other properties such as the expansion on setting. Experimental evidence showing the expansion of plaster on setting at different temperatures has recently been obtained. The observations were made incidental to the preparation of plaster samples of different pore sizes that were to be used as standard porous bodies.

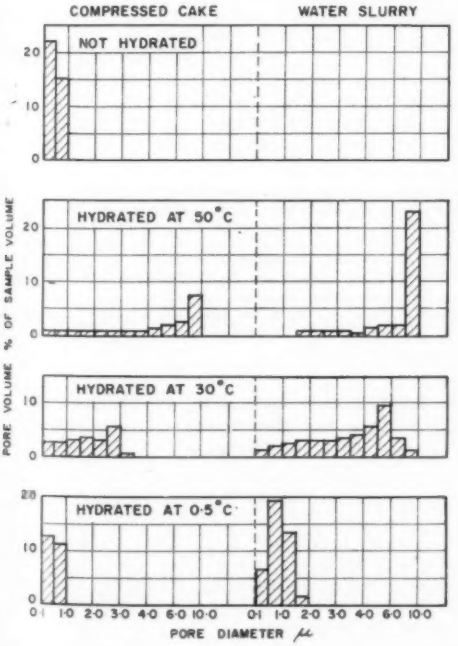


Fig. 1. Histograms for the pore-size distribution of plaster samples hydrated at different temperatures

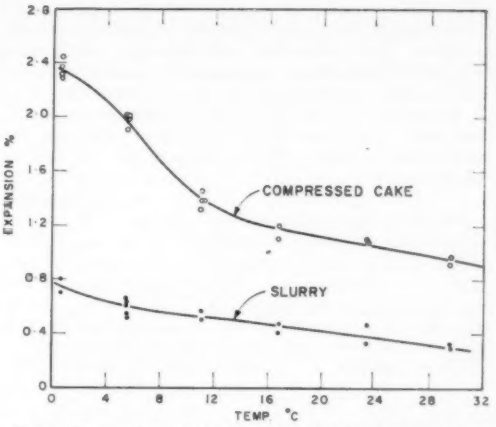


Fig. 2. Expansion on setting of plaster at different temperatures

A method was developed for compressing plaster of paris at a pressure of 5,000 p.s.i. into a cake which could then be hydrated at a desired temperature. The average pore size decreased as the temperature of set was decreased. This result confirms the observations made by Ridge[1] that crystal sizes decrease as the temperature of set is decreased.

Thus it has been found that by varying the temperature of set of plaster samples it was possible to vary their pore sizes over a wide range, as was also found by Ridge and Crook[2]. This was true whether the samples were made from a cake or water slurry of plaster of paris. Histograms for the pore-size distribution as obtained by a mercury porosimeter are given in Fig. 1. These results are for samples made from commercial unretarded plaster of paris. This plaster did not give reproducible results for expansion on setting.

Samples of pottery plaster compressed into cakes as well as mixed as water slurries in the ratio of 2 : 1 (plaster to water) were allowed to set at various temperatures. The expansion due to setting was measured with the aid of a travelling microscope. This expansion increased as the temperature of set was decreased. The results are shown in Fig. 2. The cake expanded about three times as much as the slurry for the same temperature of set.

The expansion on setting of plaster from a slurry was explained by Lea and Nurse[3] and Hill and Ridge[4] as being caused by the growth of crystals in contact. On this basis, when the conditions of setting are such as to lead to smaller crystals the chance of encounter with other crystals is greater, and expansion should be greater. It follows also that if the crystals are brought closer together initially the final expansion will be even greater for the same final size and shape of crystals. The greater expansion of the cake, which was about three times as much as that of plaster set from a water slurry at the same temperature, appears to confirm this. In the compressed cake the particles of hemihydrate are in actual contact, and expansion must begin as soon as hydration begins. In the case of the slurry, particles of hemihydrate are separated by water, and hydration can proceed for some time before encounter of the crystals occurs. In fact, a contraction is first observed when plaster begins to hydrate from a slurry.

The above results have practical significance in so far as they indicate the factors which control the expansion and pore-size distribution of setting gypsum plaster. Such information should be useful where control of these properties is essential.

I gratefully acknowledge the assistance of S. E. Dods, who carried out the laboratory experiment, and I also wish to thank B. M. O'Kelly for his helpful criticism.

P. J. Sereda

Building Materials Section,
Division of Building Research,
National Research Council,
Ottawa.

[1] Ridge, M. J., *Nature*, **182**, 1224 (1958).
[2] Ridge, M. J., and Crook, D. N., *Aust. J. App. Sci.*, **10**, 497 (1959).
[3] Lea, F. M., and Nurse, R. W., *Disc. Farad. Soc.*, **5**, 350 (1949).
[4] Hill, R. D., and Ridge, M. J., *Aust. J. App. Sci.*, **10**, 232 (1959).

Packing of Mixtures of Hard Spheres

We were pleased to see the recent communication[1] by E. B. Smith and K. R. Lea on their Monte Carlo calculation for mixtures of hard-sphere molecules. We are quite interested in this problem and have been trying to approach it experimentally by studying the random packing of mixtures of real macroscopic spheres.

When smooth spheres are poured into a container and lightly tamped, they assume a firmly packed disordered configuration. This corresponds, we believe, to the high-density limit of the disordered upper branch of the curve for the hard-sphere equation of state. In their machine computations on hard-sphere systems, Alder and Wainwright[2], and Wood and Jacobson[3], have found that an apparent phase transition to an ordered configuration usually occurs before the disordered configuration is tightly packed. By suppressing this transition, however, Jacobson and Wood were able to extend some of their Monte Carlo studies to disordered systems at the high-density limit[4]. We hope that Smith and Lea will also have an opportunity of investigating this limit in their calculations. We think it would be well worth while to check, whenever possible, how closely a few spheres with periodic boundary conditions actually behave like many spheres.

We have so far tested each of three different binary systems of 'Lucite' spheres at eleven different mixture ratios. We measure the piston displacements produced by successive pre-mixed 500-ball aliquots in each of five cylinders of various diameters. By extrapolating the average displacement volume to infinite cylinder diameter we eliminate surface effects. The results reported here have been digested from some 2,000 separate measurements.

In the three systems tested the contraction, or negative excess volume, was found to be approximately symmetrical:

$$V^{xs} \simeq -2A\ (V_1 + V_2)X_1X_2$$

where A is a dimensionless contraction factor dependent on the volume disparity, and X_1 and X_2 are 'mole' fractions. Even with our largest disparity, corresponding to a radius ratio of 1·6 : 1, there was no apparent asymmetry. By contrast, the calculations of McLellan and Alder[5] indicate a pronounced asymmetry at this radius ratio in low-density mixtures. The comparison is ambiguous, however, since their results also show that the asymmetry tends to vanish when the volume contractions are as small as those we have found.

Table 1

V_1 (c.c./ball)	V_2 (c.c./ball)	V^{xs} (x=0·5, c.c./ball)	A
0·642	0·406	−0·003 ± 0·001	0·006 ± 0·002
0·646	0·266	−0·004 ± 0·001	0·008[5] ± 0·002
0·647	0·160	−0·012 ± 0·001	0·030 ± 0·002

The actual volumes of the spheres themselves were 0·408 c.c. for the larger component and 0·260 c.c., 0·1705 c.c. and 0·1032 c.c., respectively, for the smaller components. The packing volumes for the disordered pure components, as shown in Table 1, exceed the calculated close-packed volumes by 17·0, 16·0, 15·3 and 14·8 per cent, respectively, in very good agreement with the figure of 15(±3) per cent reported by O. K. Rice[6].

The apparent variation in volume of the larger component, V_1, is an artefact of the statistical analysis. If, as we suppose, it is due to a slight unacknowledged asymmetry in V^{xs}, it should be approximately linear in the volume disparity, δ (defined below), and should affect the volumes of the smaller components in an equal but opposite manner. Our values of A should not be affected at all by such an asymmetry.

We summarize our results by:

$$A \simeq 0{\cdot}019_5\ \delta^2$$

where $\delta = 2(V_2 - V_1)/(V_2 + V_1)$ is the dimensionless volume disparity. Since A must be an even function of δ which goes to zero at $\delta = \pm 2$, as well as at $\delta = 0$, our expression gives only the limiting behaviour at small differences of radii. We find no indication of a positive V^{xs} at small δ.

For larger differences of radii there are similar results reported by C. C. Furnas on mixtures of lead shot and other systems[7]. It would be interesting to see if Furnas's estimated shrinkages for arbitrary fractional void contents in static systems are anything like the results one calculates for dynamic systems with the same voidages.

Paul C. Mangelsdorf, jun.
Elmer L. Washington

Institute for the Study of Metals,
University of Chicago,
Chicago 37, Illinois.
June 27.

[1] Smith, E. B., and Lea, K. R., *Nature*, **186**, 714 (1960).
[2] Alder, B. J., and Wainwright, T. E., *J. Chem. Phys.*, **27**, 1208 (1957).
[3] Wood, W. W., and Jacobson, J. D., *J. Chem. Phys.*, **27**, 1207 (1957).
[4] Jacobson, J. D., and Wood, W. W., Progress Report GMX-10-22, Los Alamos Scientific Laboratory (1958).
[5] McLellan, A. G., and Alder, B. J., *J. Chem. Phys.*, **24**, 115 (1956).
[6] Rice, O. K., *J. Chem. Phys.*, **12**, 1 (1944).
[7] Furnas, C. C., *U.S. Bur. Mines Bull.*, **307**, 74 (1929); also *Indust. and Eng. Chem.*, **23**, 1052 (1931).

Radioactive Labelling of Membrane Filters for the Assay of Air-borne Material

The quantitative assessment of air-borne material with membrane filters is being widely employed, mainly because of their high collection efficiency, non-hydroscopic properties and more uniform particle deposition than on other filter materials[1]. Since most of the particles deposited are held at the surface[2], the classical methods of assessment of deposits can be replaced or augmented by indirect methods. The latter consist, for example, in estimating the amount of deposit material by comparing stains[3] with suitable

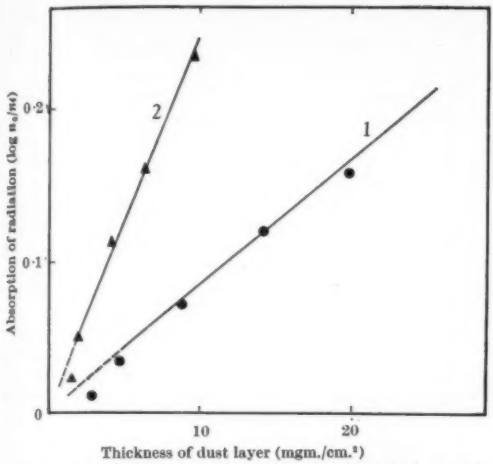


Fig. 1. Relation between β-ray absorption and thickness of dust layer. Curve 1, filters labelled with thallium-204; curve 2, filters labelled with nickel-63

standards or by objective measurement as, for example, reflectance photometry[4]. Chemical reaction between suitably impregnated or wetted filters and aerosol droplets may also be employed with quantitative estimation of the resulting colour effects[5].

It is known that various substances may be incorporated into the filter mass by adding suitable reagents to the stock solutions used for filter preparation[6]; thus it is possible to obtain filters containing chemical reagents in the form of very fine and homogeneous dispersion throughout the filter. Such reagents may react with aerosol droplets or with the surface layer of solid particles.

The application of radioactive thickness gauging for the estimation of deposit mass in filters has already been reported[7]. The present communication deals with the application of special features of membrane filters by converting the filters themselves into the radiation source used for thickness gauging, thereby eliminating the effect of filter thickness from measurement.

Although the incorporation of radioactive material into the filter mass is an evident procedure and will be reported in due course, initial work was performed by adsorbing radioactive nickel or thallium salts from solution upon the filter surface. The filters used had a mean pore size of $2r = 0{\cdot}9\mu$ and a thickness of 130μ. The diameters of the filter disks were 36 mm. Initial total filter activities were 0·1–0·2 μc. and 1–5 μc. for thallium and nickel filters, respectively.

Filters of this kind were used for the estimation of the concentration of solid aerosols and industrial dust of constant composition. Prior to air filtration, initial filter activity was measured with a Geiger–Müller window counter (3·7 mgm./cm.²) (activity n_0). Thereafter air was drawn through the filter, and as soon as a visible layer had formed on the filter, filtration was interrupted, residual activity measured and the mass of the deposit determined by weighing. This procedure was repeated several times and the expressions $\log n_0/n_i$, where n_i are the residual activities, calculated. These values were correlated with the amounts of deposit per unit filter area.

Fig. 1 illustrates a typical example with flue dust of mean particle size 1·7μ, the chief chemical components of which were alumina, ferric oxide, silica and traces of other components. Experiments summarized in Fig. 1 were performed with filters labelled with nickel and thallium.

Results indicate that the preparation of homogeneously labelled filters and measurement therewith are possible. Such filters may be used for the rapid assessment of air-borne material. The lower limit of sensitivity is at present found to be 1·6 mgm./cm.² and 6·0 mgm./cm.² for filters labelled with nickel and thallium, respectively. Because of their weak radiation and long half-lives, nickel filters seem to be the more suitable ones. With an effective filter area of 1 cm.², absolute air volumes of 10–300 litres are sufficient to obtain deposits suitable for the assessment of high and medium dust concentrations.

It is evident that the present procedure provides mass-proportional values of dust concentration. More detailed investigation indicates that the sensitivity of measurement by the method outlined depends not only on the chemical composition of the assayed dust but also on its particle size distribution.

A more detailed account of this work will appear elsewhere.

G. Kubie

Mining Institute,
Czechoslovak Academy of Sciences,
Prague, 8.

K. Spurný

Institute of Physical Chemistry,
Czechoslovak Academy of Sciences,
Prague, 12.

[1] Spurný, K., and Vondráček, V., *Chem. listy*, **50**, 1331 (1956).
[2] Kubie, G., Jech, C., and Spurný, K., *Coll. Czechoslov. Chem. Comm.* (in the press).
[3] Spurný, K., *Prac. lékarství*, **11**, 197 (1959).
[4] Paulus, H. J., Talvitie, N. A., Fraser, D. A., and Keenan, R. G. *Indust. Hyg. Quart.*, **18**, 267 (1957).
[5] Lodge, J. P., and Tufts, B. J., *Tellus*, **7**, 2 (1956).
[6] Spurný, K., *Chem. listy* (in the press).
[7] Stephan, D. G., Bohnslav, P. T., Herrick, R. A., Walsh, G. W., and Rose, jun., A. H., *Amer. Indust. Hyg. Assoc. J.*, **19**, 276 (1958).

RADIATION CHEMISTRY

Changes in Conductivity of Pure Water caused by X-Irradiation

Most of our present knowledge about the physico-chemical processes occurring in the radiolysis of water has been obtained by purely chemical methods[1]. I have now tried to obtain some additional data by using a physical method. In the course of the primary and secondary processes which occur when water is irradiated with ionizing radiation, it is possible that charged particles will be formed which cause an increase in conductivity during irradiation. I therefore studied the variation with time of the conductivity of pure, non-degassed water when it was submitted to pulsed X-irradiation, in order to obtain information about the eventual concentration and life-time of such charged particles[2].

In a preliminary investigation, water, prepared by distilling three times—twice in a quartz still—with access of air, was irradiated in a conductivity cell made of 'Plexiglas' and with silver electrodes, with X-ray pulses (60 kVp., average dose-rate about 5 kr./min.) obtained by a rotating sectored disk.

A d.c. voltage was applied to the cell through a resistor, so that a periodical change in conductivity synchronous with the X-ray pulses would give rise

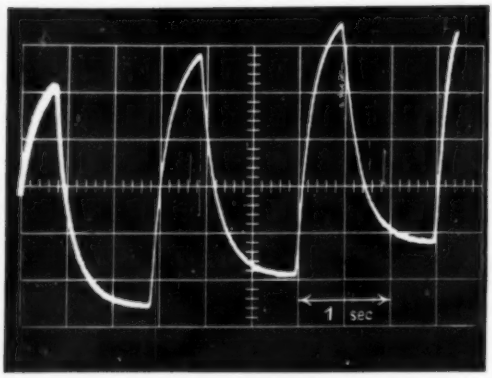


Fig. 1. Photograph of an oscilloscope picture showing the variation of conductance when water is irradiated with square wave X-ray pulses (light to dark period 1 : 2)

to an a.c. voltage with the same frequency. This voltage was measured by means of a synchronized amplifier. The synchronization was effected by interrupting the light beam falling on a photoelectric cell by the same sectored disk.

The experiment showed that there was in fact a periodical change in conductivity, the amplitude of which was inversely proportional to pulse frequency in the range from 10 to 3,000 c.p.s. covered in this investigation. It could therefore be concluded that during irradiation ions were formed with a life-time greater than 0·1 sec.

Based on these results, I constructed an apparatus with which I could display on an oscilloscope screen the variation with time of the conductivity of water irradiated with slow X-ray pulses (0·2–20 c.p.s.).

The water used in this experiment was pre-distilled first from alkaline permanganate and then from acid dichromate solution before being further purified in the quartz still, in order to be certain that no organic impurities were present. Between the two quartz distillation stages the vapour was heated to about 800° C. The conductivity cell, this time made of quartz and with platinum electrodes, was connected to an a.c. bridge operating at 2 kc.p.s. The diagonal voltage, which was proportional to the variation of conductance, was amplified, rectified, and fed to a d.c.-oscilloscope ('Tektronix').

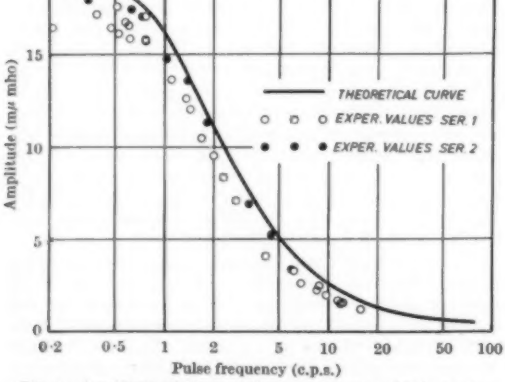


Fig. 2. Amplitude of the periodic variation of conductance as a function of pulse frequency. The theoretical curve is calculated from the experimentally obtained dose-rate dependence and life-time. Maximum dose-rate, 11·9 krads/min.

Fig. 1 shows a photograph of the oscilloscope picture showing the variation of conductance caused by irradiation with square wave-pulses (light to dark period = 1 : 2). In Fig. 2 the amplitude of the periodical conductance changes is plotted against pulse frequency. Two effects seem to be superimposed: (1) A reversible increase in conductivity during irradiation which returns to zero after the end of irradiation; (2) an irreversible rise in conductivity which starts practically instantaneously with irradiation and continues until the end of irradiation.

An analysis of the variation of conductivity as a function of pulse-frequency and dose-rate leads to the conclusion that the reversible effect may be due to the formation of ions the concentration of which varies according to the differential equation:

$$\frac{d[X]}{dt} = G_x \frac{\delta}{N_a} - k'[X][S] - 2k[X]^2$$

where $[X]$ is the concentration of the ions in question, $[S]$ that of a scavenger substance with which these ions can react monomolecularly, N_a the Avogadro number, δ the dose-rate, k and k' reaction constants, and G_x the yield of the ions formed.

A comparison of the properties of the observed ions with radiation-chemical data from the literature suggests that the ion X may be identical with the radical-ion $\cdot O_3^-$, that is, the anion of the radical $HO_2\cdot$ (ref. 3). If this is assumed and if the radical $HO_2\cdot$ is completely dissociated at the pH of the water investigated (5·5): $G_x = 4{\cdot}3$ radicals/100 eV., $k'[S] = 2{\cdot}9$ sec.$^{-1}$, and $k = 1{\cdot}45 \times 10^9$ litres/mole × sec. The radical-ion $\cdot O_2^-$ is, according to Haïssinsky[4], formed directly by the reaction of H-radicals with the dissolved oxygen:

$$H\cdot + O_2 \rightarrow H^+ + \cdot O_2^-$$

A more detailed discussion of a chemical reaction mechanism suggested leads to the equation: $G_x = G_H + G_{OH} + G_{HO_2} - 0{\cdot}4 = 4{\cdot}6$ radicals/100 eV. (with radical yields according to Allen and Schwarz[5]). Dainton and Rowbottom[6] have determined the recombination constant of the radical $HO_2\cdot$ as 3×10^9 litres/mole × sec. The difference between this value and mine (for $\cdot O_2^-$) might be explained by a difference in pH.

The irreversible effect observed in my experiment is probably due to the formation of percarbonic acid from the carbon dioxide dissolved in the water[7]. According to my experiment the yield of this reaction would be $G(H_2CO_4) = 0{\cdot}2$ molecule/100 eV. The term 0·4 in the equation for G_x mentioned above is twice this value.

A full account of this investigation will be published elsewhere.

K. Schmidt

Max Planck-Institut für Biophysik,
Forsthausstr. 70,
Frankfurt a. M.

[1] Dainton, F. S., *Brit. J. Radiol.*, **31**, 645 (1958).
[2] Lotz, A. P., and Schmidt, K., *Strahlentherapie*, **110**, 223 (1959).
[3] Weiss, J., *Trans. Farad. Soc.*, **31**, 668 (1935).
[4] Halssinsky, M., *J. chim. phys.*, **53**, 542 (1956).
[5] Allen, A. O., and Schwarz, H. A., Proc. Internat. Conf. Geneva (1958).
[6] Dainton, F. S., and Rowbottom, J., *Trans. Farad. Soc.*, **49**, 1160 (1953).
[7] Günther, P., and Holzapfel, L., *Z. Phys. Chem.*, **49**, 303 (1941).

Radiation-induced Nitration of Aliphatic Hydrocarbons

THE behaviour of fission product iodine in a tributyl phosphate–kerosene system for reprocessing pile-irradiated uranium fuels has recently been studied in this laboratory. It was found that the principal mode of fixation of iodine in the hydrocarbon diluent was scavenging by hydrocarbon radicals produced by the action of the fission product radiation. However, it was observed that the G-value for fixation under process conditions was only about 0·1. This is very much lower than the values of about 7·0 reported for air-free n-heptane[1] and 5·6 for air-free *cyclo*hexane[2], which are generally accepted as giving some indication of the total yield of radicals in irradiated aliphatic hydrocarbons.

It was considered that other radical scavengers must be present in the process solutions in addition to the iodine. Atmospheric oxygen will certainly be at least partly responsible for the low uptake of iodine; but it was surmised that the nitrogen oxides, always present in radioactive nitrate solutions, may be more important in some cases. The oxides NO and NO_2 both have an 'odd electron' structure and are paramagnetic; they would therefore be expected to combine with alkyl radicals rapidly and with little activation energy requirement. To test this idea, n-dodecane containing 10 gm. NO_2/litre was irradiated using a 7·2-c. strontium-90 β-source. The mean radiation dose-rate was 2×10^{18} eV./ml. hr. and the duration of the irradiation was 114 hr. No special precautions were taken to exclude air. Infra-red spectral examination of the irradiated dodecane showed strong R—NO_2 (1,555 cm.$^{-1}$, 1,350 cm.$^{-1}$) and R—COOH (1,720 cm.$^{-1}$) bands. Slight absorption peaks at 1,680 cm.$^{-1}$ and 1,645 cm.$^{-1}$ were attributed to alkyl nitrite and alkyl nitrate, respectively. Comparison with standard substances showed that the G-value for R—NO_2, assumed to be nitrododecane, was 5·0. The yield of carboxylic acid, taken as dodecanoic acid, was about 1·0. No change could be detected in a dodecane solution of NO_2 which had been allowed to stand in the absence of radiation for a similar length of time. Carboxylic acid formation is presumably the end result of radical scavenging by atmospheric oxygen: in an earlier experiment, in which the dissolved NO_2 became exhausted, the yield of carboxylic acid was more than doubled.

These results are in good agreement with previous work on iodine scavenging in oxygen-free hydrocarbons[1,2] and suggest that one of the most important solvent degradation phenomena in highly active tributyl phosphate–hydrocarbon processes may be radiation-induced nitration. It is probable, in NO_2 solutions that are completely devoid of oxygen, that the only products arising from the alkyl radicals will be nitroalkanes together with small amounts of alkyl nitrites, the latter possibly arising from a reaction of alkyl radicals with excited NO_2 molecules:

$$R + NO_2 \rightarrow R{-}N\begin{matrix}=O\\ \searrow O\end{matrix}$$

$$R + NO_2^* \rightarrow R{-}O{-}N{=}O$$

The radiation chemistry of aliphatic hydrocarbon–nitrogen oxide systems seems to be worthy of further study since information thus obtained should be of considerable value in elucidating organic radiation chemical mechanisms in general, and would provide a unique opportunity of obtaining unequivocal evidence regarding some of the fundamental processes proposed in contemporary theories of aliphatic nitration such as, for example, those reviewed by A. V. Topchiev[3].

I am indebted to Mr. E. Hughes, of the Chemical Services Department, Windscale, for the infra-red analysis involved in this work.

This communication is published by permission of Sir William Cook, managing director, and Dr. H. Kronberger, director of research and development, of the Development and Engineering Group of the United Kingdom Atomic Energy Authority.

TYSON RIGG*

U.K. Atomic Energy Authority,
Development and Engineering Group,
Windscale Works,
Sellafield,
Seascale,
Cumberland.

* Present address: Research Council of Alberta, Edmonton, Alberta.

[1] Forsyth, P. F., Weber, E. N., and Schuler, R. H., *J. Chem. Phys.*, **22**, 66 (1954).

[2] Fessenden, R. W., and Schuler, R. H., *J. Amer. Chem. Soc.*, **79**, 273 (1957).

[3] Topchiev, A. V., "Nitration of Hydrocarbons" (Pergamon Press, London, 1959).

Role of Oxygen in the Cross-linking and Degradation of Deoxyribonucleic Acid by Ionizing Radiations

WHEN deoxyribonucleic acid in dilute aqueous solutions is irradiated with X-rays, the attack by the free radicals produced in water leads to main-chain scission, and this reaction is essentially the same in the presence as in the absence of oxygen[1]. Irradiation of nucleic acid in the solid state and containing less than 25 per cent of water leads to a complex series of reactions which, judged by physico-chemical measurements, were largely unaffected by oxygen. With nucleic acid gels having a water content between these two extremes, oxygen was found to exert a pronounced influence on the changes produced by irradiation with 1-MeV. electrons. Since deoxyribonucleic acid is present in the cells in the form of a relatively concentrated gel, the response to radiation in this form is more likely to be of biological significance than the behaviour of dilute solutions or nearly dry solid.

The nucleic acid used in these gel experiments was obtained from salmon sperm, contained little salt (less than 3 per cent sodium acetate) and was free from protein (less than 0·02 per cent). Its molecular weight was 10^7 and its physico-chemical properties have been described in detail (see ref. 2). The molecular weight (determined by light-scattering) of nucleic acid in the dry or nearly dry state initially falls on exposure to 1-MeV. electrons to about two-thirds of its starting value and then increases again, while the intrinsic viscosity falls continually with dose. From these and other data, Alexander, Lett, Moroson and Stacey[2] concluded that irradiation produced

branching and main-chain scission simultaneously to approximately the same extent, so that the molecule becomes less asymmetric (that is, resulting in a fall of viscosity) without there being a substantial change in the average molecular weight.

By equilibrating the nucleic acid with air or nitrogen at different relative humidities, the water content (gm. of water per 100 gm. of polymer) was varied from $< 0{\cdot}5$ per cent to 200 per cent. As the water content is increased above 25 per cent, main-chain scission with reduction in molecular weight becomes the predominant reaction of irradiation in air (see Fig. 1). When the gels are irradiated in the absence of oxygen, the molecular weight increases (see Fig. 1) until some of the nucleic acid has become so highly cross-linked that it no longer dissolves in dilute salt solution, but only swells. Fig. 2 shows that the formation of this insoluble network has a threshold, and the behaviour of 'wet' nucleic acid irradiated in the absence of air is typical of a cross-linking system such as polythene[4]. Once cross-linked gel begins to be formed, the molecular weight (because of polydispersity, it is probable that the molecular weights of the highly cross-linked samples are underestimated) of the soluble fraction falls because the largest units are preferentially linked into the insoluble fraction. This again is in accord with polymer theory[4]. The quantitative recovery of the insoluble nucleic acid is difficult, since after suspension in dilute salt solution much of it is present in a highly swollen form which can only be removed by high-speed centrifugation and the values obtained vary somewhat from experiment to experiment.

We interpret these results, which will be published in full elsewhere, by assuming that sparsely ionizing radiations produce reactive centres in the nucleic acid molecules which can combine with one another to give cross-links, or react with oxygen to give a product which can no longer develop cross-links (cf. ref. 5). The increase in the number of cross-links formed in the absence of oxygen by a given dose with

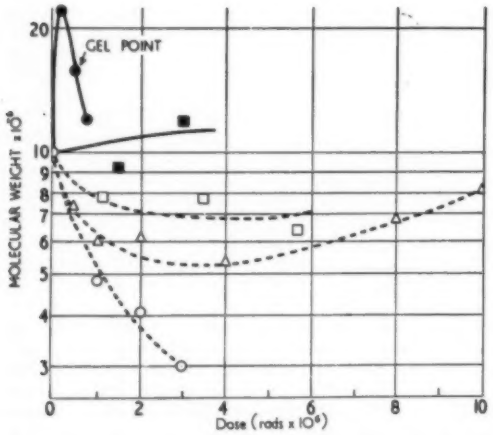


Fig. 1. Change in average molecular weight (measured by light scattering) of deoxyribonucleic acid fibres with different moisture contents after irradiation with 1-MeV. electrons under air and nitrogen

○, 100 per cent water content irradiated in air
●, 100 „ „ „ „ „ „ nitrogen (or vacuum)
△, 20 „ „ „ „ „ „ air
□, 0 „ „ „ „ „ „ air
■, 0 „ „ „ „ „ „ nitrogen (or vacuum)

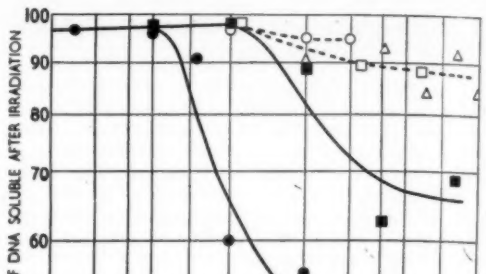


Fig. 2. Fraction of deoxyribonucleic acid rendered insoluble in *M*/100 sodium chloride (that is, removed by 6 hr. centrifugation at 20,000*g*). Symbols as in Fig. 1

increasing water content of the nucleic acid specimen we attribute to the greater mobility in wet samples of the polymer chains, and this promotes interaction between the active points. In nucleic acid with less than 25 per cent of water, inability of active centres to meet hinders cross-linking.

In the presence of air, few cross-links are formed by direct action, and the degradation observed on irradiation of the samples containing an equal weight of water is due to direct action plus the attack by the radicals formed in the water. In the absence of oxygen, the active centres produced by direct action give rise to cross-links which exceed the number of main-chain scissions from the combination of direct and indirect actions, and therefore the net effect is an increase in molecular weight and gellation. Because the indirect effect produces only scission, there must, at a given dose under nitrogen, be a water content at which the amounts of scission and cross-linking are equal and above which gel is not formed. For a dose of 10^6 rads this occurs at 300 per cent of water.

We wish to thank Mr. D. Moore of the Medical Research Council Experimental Radiopathology Unit for radiation facilities.

This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

Peter Alexander
J. T. Lett

Chester Beatty Research Institute,
Institute of Cancer Research,
Fulham Road,
London, S.W.3.

[1] Smith, D. B., and Butler, G. C., *J. Amer. Chem. Soc.*, **73**, 258 (1951). Conway, B. E., *Brit. J. Radiol.*, N.S., **27**, 42 (1954).
[2] Lett, J. T., and Stacey, K. A., *Makromol. Chem.*, **38**, 204 (1960).
[3] Alexander, P., Lett, J. T., Moroson, H., and Stacey, K. A., *Int. J. Radiation Biology*, Supp. 1, 47 (1959).
[4] Charlesby, A., *Proc. Roy. Soc.*, A, **222**, 60 (1954).
[5] Alexander, P., and Charlesby, A., *J. Pol. Sci.*, **23**, 355 (1957).

CHEMISTRY

Possible Discovery of Ion-Pairs by Interferometry

In the course of an investigation on electrodeposition to determine the cause of what Ibl and Muller[1] called an "anomalous maximum" near the cathode, and which O'Brien and Axon[2] independently noted and named the "second wave" in a complete, electrode-to-electrode interference pattern, it was found that this second wave does not develop in electrolyte solutions in which ion-pairs do not form. Such a solution is nickel sulphate[3], in which it has been shown that, presumably because of the strength of the bonds and lack of lability in the octahedral nickel aquo-complex, the closest approach of the sulphate ion cannot be less than the diameter of one water molecule (see Figs. 1 and 2). Electrolysis of a silver perchlorate solution gave the faintest traces of a second wave, which may be considered to be additional supporting evidence. Since Fajans and Luhdemann[4] have shown that free ions in solution may be expected to give higher refractive indices than ion pairs, and indeed a plot of concentrations versus refractive index of values taken from Landolt–Bornstein shows no deviation from linearity for nickel sulphate (which deviation is usually attributed to ion-pairs) until concentrations in the neighbourhood of 1 *M*, it follows that the cathode maximum may be explained by the dissociation of ion-pairs.

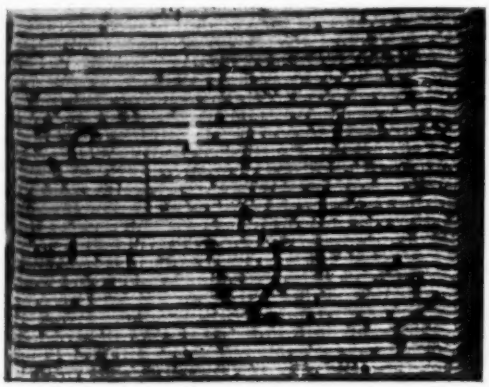


Fig. 1. 30 gm./l. copper sulphate, 3 mm. electrode separation, 2 m.amp./cm.², 10·8° C., 0·19700 V.

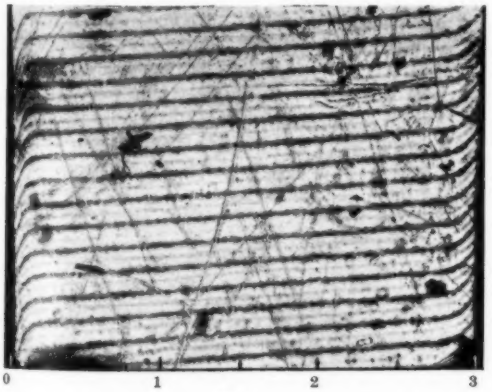


Fig. 2. 33 gm./l. nickel sulphate, 3 mm. electrode separation, 3 m.amp./cm.², 25·0° C., 2·0 V.

Fig. 3. 30 gm./l. copper sulphate, 0·2 mm. electrode separation, 2 m.amp./cm.², 9·8° C., 0·17221 V.

It was considered that the formation of ion-pairs was probably not an instantaneous process and that the electrodeposition cell being used was suited to a study of the kinetics of this process. The cell contains about 0·15 ml. of solution when the depth of the electrodes is 2 mm. and their separation 3 mm.

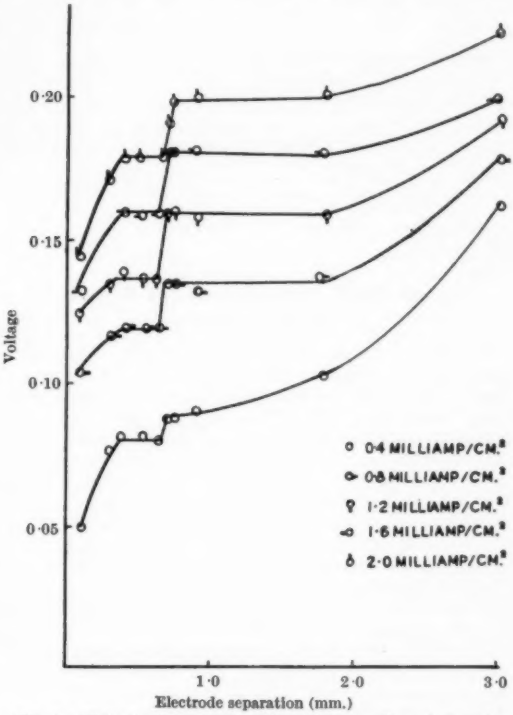


Fig. 4. Plot of voltage against electrode separation at several current densities

It can be calculated from the mobility of the ion (only Cu^{2+} need be considered as the cell is $Cu|CuSO_4|Cu$), which is about $1{\cdot}12 \times 10^{-3}$ cm./sec., that at 0·33 V. the time to traverse the 3 mm. of the cell is about 300 sec. The maximum of the second wave occurs at about 0·1 mm. from the electrode at both electrodes, and its amplitude has been found to vary directly as the current density[2], and the 'time of migration' to this distance should be 9 sec. Hence when the electrodes are placed so that the anode and cathode second waves overlap (Fig. 3), no second wave should form, and this was found to be so. But since there is the possibility that the effective dielectric constant of the solution is low and a change in field gradient might be expected, we do not place any special significance on the time of 9 sec.

When the electrodes were moved together at constant current density, a family of curves (Fig. 4) was found. The curves have the same features occurring at the same distance. A plateau where there is no apparent increase in total resistance with distance begins at about 0·37 mm. and ends at about 0·7 mm. in an abrupt, nearly vertical step.

It was expected that the distance at which the first plateau was found would vary with the voltage applied, since conventionally the speed of the ions has been considered to depend on this. This effect has not been found. The step height varies around about 0·02 V. while the expected height by Nernst's equation and thermodynamics from the dissociation constant of copper sulphate is 0·06 V.

Explanation of these and other aspects is being actively investigated, and greater precision of measurement is being sought.

R. N. O'Brien
C. Rosenfield

Department of Chemistry,
University of Alberta,
Edmonton.

[1] Ibl, N., and Muller, R. H., *Z. Elektrochem.*, **59**, 671 (1955).
[2] O'Brien, R. N., and Axon, H. J., *Trans. Inst. Met. Finishing*, **34**, 4 (1957).
[3] Duncan, J. F., and Keppert, D. L., in "The Structure of Electrolyte Solutions", edit. by Hamer, 398 (John Wiley and Sons, New York, 1959).
[4] Fajans, K., and Luhdemann, R., *Z. phys. Chem.*, B, **29**, 150 (1935).

Solid-Solid Interactions on Active Adsorbents

In recent years the diffuse spectral reflectance properties of various solid systems have been investigated[1,2], and several analytical applications have been developed[3-5]. Often it has been reported advantageous to mix the optically absorbing substance with an inactive powdered diluent such as sodium or potassium fluoride. When this is done the reflectance spectrum of the absorbing substance is sharpened and clarified in many cases. More pronounced effects have been obtained when a so-called active adsorbent powder such as chromatographic alumina or silica gel is substituted for the inactive diluent. In an attempt to study the nature of the changes brought about by active adsorbents in the solid state, pilot experiments have been carried out using Michler's ketone (4,4′-*bis*dimethylaminobenzophenone) mixed with active alumina. Michler's ketone has a well-defined absorption spectrum in transmittance in solution and reflectance from the

Fig. 1. Changes with time in the absorbance at the wave-length of the maxima of various mixtures of Michler's ketone and chromatographic alumina as measured by reflectance. 1, 2 mgm. ketone and 3 gm. acidic alumina; 2, 1 mgm. ketone and 3 gm. acidic alumina; 3, 2 mgm. ketone and 3 gm. basic alumina; 4, 1 mgm. ketone and 3 gm. basic alumina

solid, and is quantitatively removed from solution in a non-polar solvent by alumina. In a typical experiment 1 and 2 mgm. of the powdered ketone were mixed with 3 gm. of acidic and basic alumina (Woelm) and the solid mixtures allowed to stand for several days at room temperature in sealed containers. Visual changes slowly occurred in these systems until in appearance they were virtually identical with alumina on which the ketone had been adsorbed from a solution as in ordinary column chromatography. It is suggested that the two phenomena involve essentially the same adsorption process, differing only in rate.

The diffuse reflectance spectra of the Michler's ketone–alumina mixtures were measured at intervals as the solid–solid interactions were taking place. The instrument used was a Beckman model *DU* spectrophotometer with a standard reflectance attachment. Regular changes with time, both in the total absorbance (log base 10 of the reciprocal reflectance) and in the wave-length of the absorbance maxima (Figs. 1 and 2), were observed. Throughout, the gross form of the spectra remained characteristically

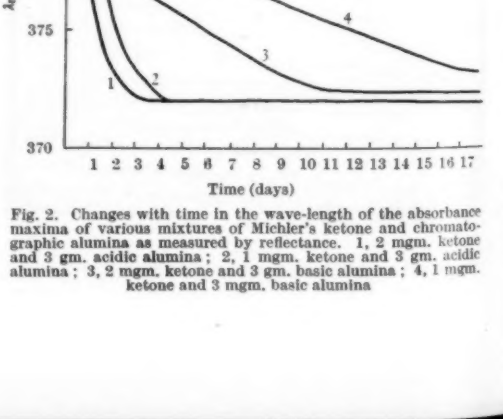


Fig. 2. Changes with time in the wave-length of the absorbance maxima of various mixtures of Michler's ketone and chromatographic alumina as measured by reflectance. 1, 2 mgm. ketone and 3 gm. acidic alumina; 2, 1 mgm. ketone and 3 gm. acidic alumina; 3, 2 mgm. ketone and 3 gm. basic alumina; 4, 1 mgm. ketone and 3 mgm. basic alumina

that of the ketone. The displacement of the absorbance maxima should be related to distortions in the electronic energy-levels of the ketone molecules due to interactions with the alumina on adsorption. The changes in absorbance at the wave-length of the maxima for the adsorbed species should be an index of the relative amount of the adsorbed species present at a given time. Further, the rates at which adsorption takes place on the different grades of alumina provide a measure of the relative activities of such adsorbents. When the solid–solid adsorption processes were essentially complete, the absorbance maxima were virtually identical with those of the ketone adsorbed on the same types of alumina from solutions. This confirms the visual impression as to the similarity of at least the end-result of these two processes. The adsorption from solution, of course, occurs far too rapidly to be studied kinetically, while the solid–solid process proceeds at a vastly retarded but measurable rate. It is hoped that the data being accumulated by reflectance will contribute to the knowledge of solid adsorptions and perhaps submit to a kinetic analysis which could help suggest the mechanism involved. A detailed account of this work will be published separately.

The financial support received from a Frederick Gardner Cottrell grant of the Research Corporation of New York is gratefully acknowledged.

PHILIP ANTHONY
HARRY ZEITLIN

Chemistry Department,
University of Hawaii,
Honolulu 14, Hawaii.

[1] Griffiths, T. R., Lott, K. A. E., and Symons, M. C. R., *Anal. Chem.* **31**, 1338 (1959).
[2] Zeitlin, H., and Niimoto, A., *Anal. Chem.*, **31**, 1167 (1959).
[3] Naughton, J. J., Frodyma, M. M., and Zeitlin, H., *Science*, **125**, 121 (1957).
[4] Naughton, J. J., Zeitlin, H., and Frodyma, M. M., *J. Agric. Food Chem.*, **6**, 933 (1958).
[5] Shibata, K., *J. Biochem.* (Japan), **45**, 599 (1958).

Hydrocracking of Neopentane and Neohexane over Evaporated Metal Films

FOR the cracking of ethane in the presence of hydrogen over iron[1] and over nickel[2], it has been supposed that the molecule is adsorbed at adjacent carbon atoms (1–2 diadsorbed) prior to cracking. However, since work in this laboratory on the hydrocracking of higher hydrocarbons has given results which cannot readily be explained by 1–2 diadsorbed intermediates, the behaviour of neopentane and neohexane was investigated in order to establish the possibility of alternative mechanisms. In the case of the former, 1–2 diadsorption is impossible while in the latter it must be limited to the ethyl group attached to the quaternary carbon atom.

Table 1 shows results of reactions over evaporated films of nickel, tungsten, platinum and rhodium, using initial pressures of hydrogen of 45 mm. mercury and of hydrocarbon 3·7 mm. mercury. The metal films were prepared from spectroscopically standardized metals. Analyses were made after 5–25 per cent of the hydrocarbon had reacted, using liquid-modified gas adsorption chromatography[3]. The column, 60 ft. in length, with a squalane-on-'Pelletex' (a pelleted carbon black) packing, operating at 35° C., was capable of separating all saturated hydrocarbons from C_1 to C_6. The limit of detection was about 10^{-6} mole, equivalent to a pressure of 3×10^{-2} mm. mercury in the reaction vessel.

The results in Table 1 show that hydrocracking of neopentane and of propane occurred over nickel, tungsten and platinum with comparable ease, whereas neohexane reacted at temperatures up to 50 deg. C. below those for neopentane. Over nickel, hydrocracking of ethane required a temperature about 40 deg. C. above that for propane, and the overall activation energies for hydrocracking are 35 ± 5 kcal. mole^{-1} for both neopentane and propane and 69 kcal. mole^{-1} for ethane. These values may be compared with 34 and 54 kcal. mole^{-1} for propane[4] and ethane[2] respectively, obtained on nickel–kieselguhr catalysts using rather lower hydrogen-to-hydrocarbon ratios and lower temperatures. The order of catalyst reactivity for hydrocracking at the quaternary carbon atom is rhodium > tungsten > nickel > platinum, the same order as for propane. This order is quite different from that for the breaking of the C—N bond in methylamine and ethylamine, which is rhodium ≃ platinum > nickel > tungsten[5]. The reaction of neopentane on platinum is remarkable since the presence of 17 per cent *n*-butane in the product indicates substantial isomerization as well as cracking.

In the reactions of neohexane, cracking at the quaternary carbon atom to give *iso*butane and ethane as primary products predominated strongly on platinum and decreased in importance in the order platinum > tungsten ≫ nickel > rhodium, so that for rhodium the dominant initial cracking mode was in the ethyl group to yield methane and neopentane.

Table 1

Catalyst	Hydrocarbon	Temp. (° C.)	Rate (mm. per hr. per 10 mgm. of catalyst)	Extent of reaction (per cent)	Distribution of product (per cent)*						
					M	*E*	*P*	*iso-B*	*n-B*	*neo-P*	*iso-P*
Nickel	Neopentane	230	0·3	8	95	4	1				
	Neohexane	188	0·4	10	46	9	5	13		27	
	Ethane	272	0·3	8	100						
	Propane	230	0·7	10	89	11					
Tungsten	Neopentane	202	0·07	2	57	17	11	15			
	Neohexane	161	0·2	7	33	19	6	36		6	
	Propane	188	0·7	25	65	35					
Rhodium	Neopentane	157	0·9	24	73	18	4	5			
	Neohexane	105	0·8	13	46	1	1	4		37	11
Platinum	Neopentane	296	0·4	11	25	14	17	27	17		
	Neohexane	285	0·4	10	5	41	5	44		5	
	Propane	295	0·5	10	40	60					

* *M*, methane; *E*, ethane; *P*, propane; *iso-B*, *iso*butane; *n-B*, *n*-butane; *neo-P*, neopentane; *iso-P*, *iso*pentane.

These results show that the claim of Haensel and Ipatieff[6] that, over a nickel/kieselguhr catalyst, bonds to a quaternary carbon atom are least susceptible to attack, is not of general validity for other catalysts. Furthermore, in the hydrogenation of 1–1 dimethyl-*cyclo*propane on nickel–kieselguhr at 150° C. (ref. 7), the sole product, *iso*pentane, indicates exclusive rupture of a bond to the quaternary carbon atom. In this case, however, the highly strained *cyclo*propane ring may invalidate direct comparison with the saturated hydrocarbons.

Although these results show that hydrocracking of a C—C bond can occur without 1–2 diadsorption, it does not follow that the adsorbed intermediate is attached to the catalyst at only one carbon atom. There is evidence for 1–3 diadsorption of neopentane in catalytic exchange with deuterium[8]. In the diadsorbed intermediates responsible for multiple exchange between deuterium and saturated hydrocarbons (cf. Anderson[9]), each carbon atom is normally linked to the surface by a single bond, and the 1–2 diadsorbed intermediate previously postulated for the cracking of ethane[1,3] is identical with that established for multiple exchange in ethane[10]. However, assuming a value of ΔH for adsorption by a single bond of -12 kcal. mole^{-1}, and using corresponding characteristic temperatures for exchange[9] and cracking, it follows that the average life-time of such a diadsorbed species will, at cracking temperatures, be only 10^{-3} to 10^{-4} of the life-time under exchange conditions. Furthermore, it is known that over both nickel and tungsten an increase in temperature favours the further loss of hydrogen atoms from the adsorbed hydrocarbon residue[11]: the reduced rate of exchange of neopentane[8] and ethane[10] over nickel at higher temperatures is probably due to the formation of strongly adsorbed species, multiply bonded to the surface. It seems unlikely, therefore, that the same diadsorbed species is the principal intermediate in both exchange and hydrocracking. We suggest, at least in hydrocracking reactions where 1–2 diadsorption is sterically impossible, that reaction proceeds via 1–3 diadsorbed species and that, in general, diadsorbed intermediates in hydrocracking reactions are comparatively strongly adsorbed on the catalyst surface by the loss of more than one hydrogen atom on at least one carbon atom.

We are grateful to the Shell Co. (Australia), Ltd., for a grant towards the cost of materials.

J. R. Anderson
B. G. Baker

Department of Chemistry,
University of Melbourne.

[1] Cimino, A., Boudart, M., and Taylor, H. S., *J. Phys. Chem.*, **58**, 796 (1954).
[2] Kemball, C., and Taylor, H. S., *J. Amer. Chem. Soc.*, **70**, 345 (1948).
[3] Eggertsen, F. T., Knight, H. S., and Groennings, S., *Anal. Chem.*, **28**, 303 (1956).
[4] Morikawa, K., Trenner, N., and Taylor, H. S., *J. Amer. Chem. Soc.*, **59**, 1103 (1937).
[5] Kemball, C., and Moss, R. L., *Proc. Roy. Soc.*, A, **224**, 398 (1958).
[6] Haensel, V., and Ipatieff, V. N., *Indust. Eng. Chem.*, **39**, 853 (1947).
[7] Slobodin, Ia. M., Grigor'eva, V. I., and Shmuliakovskii, Ia. E., *J. Gen. Chem.* (*U.S.S.R.*), **23**, 1480 (1953).
[8] Kemball, C., *Trans. Farad. Soc.*, **50**, 1344 (1954).
[9] Anderson, J. R., "Reviews of Pure and App. Chem.", **7**, 165 (1957).
[10] Anderson, J. R., and Kemball, C., *Proc. Roy. Soc.*, A, **223**, 361 (1954).
[11] Wright, P. G., Ashmore, P. G., and Kemball, C., *Trans. Farad. Soc.*, **54**, 1692 (1958). Galwey, A. K., and Kemball, C., *ibid.*, **55**, 1959 (1959).

BIOCHEMISTRY

Assay of Corticosteroids in the Chick Embryo

For some time we have been studying various manifestations of the reaction induced in chick embryos by the inoculation of leucocytes from normal fowls (the Simonsen phenomenon) with special interest in the production of macroscopic foci on the chorioallantois[1]. In the course of this work it was observed that if hydrocortisone was added to the inoculum the number of foci was diminished and the embryo showed obviously retarded development.

Several studies of the effect of cortisone on the developing chick embryo have been reported[2–4]. They showed a moderate inhibition of growth with doses of the order of 0·3–0·5 mgm. per embryo. We have not found any reports of such studies with the newer types of anti-inflammatory corticosteroids.

Through the co-operation of Dr. T. F. McCrae of Glaxo Laboratories we have been able to test the following: cortisone alcohol, hydrocortisone alcohol, prednisone alcohol, prednisolone disodium phosphate and dexamethazone. All were used as suspensions in normal saline with the exception of prednisolone disodium phosphate which is freely soluble in water. In order to prevent any damage to the area used for inoculation of leucocytes, the suspensions were inoculated in 0·1 ml. vol. into the air space of twelve-day embryos and the eggs held vertically in the incubator for 1–2 hr. before being inoculated on the dropped chorioallantois with $2–5 \times 10^5$ fowl leucocytes in a volume of 0·1 ml. After four days further incubation at 38° C. the embryos were removed and weighed and the foci on the chorioallantoic membranes counted.

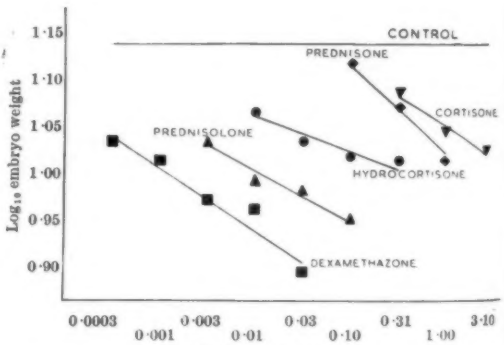


Fig. 1. Action of corticosteroids on embryo weights. Doses are shown in mgm./embryo, logarithmic intervals being used. The values have been adjusted to a uniform control weight of 13·8 gm.

The results for the significant range of doses of the five compounds used are shown in Fig. 1. Each point is the mean of a group of 8–16 embryos except for the highest doses of prednisolone and dexamethazone where some embryos died before the fourth day. The survivors from the larger doses of the 11-hydroxysteroids usually showed, in addition to retarded growth, definite pathological changes in the form of œdema, liver damage and abnormalities of feather growth. This was not seen with prednisone, where the effect seemed to be a simple retardation of growth.

The effect on focal counts from a standard inoculum of adult fowl leucocytes followed a similar trend. In

Table 1. Action of Corticosteroids in Reducing Focal Count on Chorioallantoic Membrane

Dose mgm. embryo	Prednisone per cent	P	Hydrocortisone per cent	P	Prednisolone per cent	P	Dexamethazone per cent	P
0·0003		—		—		—	40	0·02
0·001		—		—		—	10	<0·01
0·003		—		—	90	0·35	7	<0·01
0·01		—	35	0·05	30	0·01	5	<0·01
0·03	70	0·10	30	0·02	5	<0·01	0	<0·01
0·10	50	0·01	20	<0·01	2	<0·01		—
0·31	50	0·01	5	<0·01		—		—
1·00	30	<0·01		—		—		—

All values are based on the median of the focal counts and indicate percentage of the corresponding control counts.

all experiments on randomly bred embryos there is a wide range of counts and the median has been taken as the most useful index of comparison. In Table 1 the medians have been adjusted as percentages of the corresponding control values and the statistical significance of their difference from the control shown by the value p as calculated from Student's test. When cortisone or prednisone was given by the air space no effect on focal count was observed and the values shown are from an experiment in which prednisone was mixed with the leucocytes just prior to their inoculation.

It will be seen that by both criteria the activities of the corticosteroids are in the same order:

dexamethazone > prednisolone > hydrocortisone > prednisone, cortisone

The striking difference between prednisone and prednisolone, which by the conventional tests in laboratory mammals are of approximately equal activity, is noteworthy and may reflect the absence of some enzymic capacity in the embryos.

These experiments are being extended to other corticosteroids, and exploratory work on the nature of the action of prednisolone on the focal count is in progress.

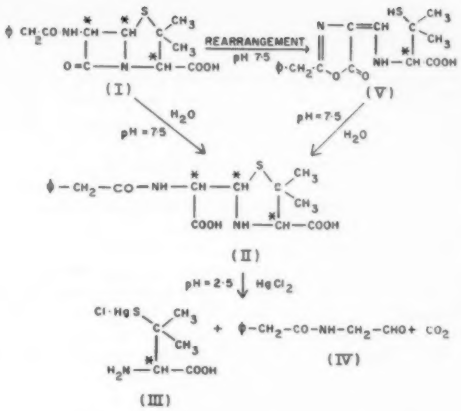
F. M. Burnet
N. L. Warner

The Walter and Eliza Hall Institute of Medical Research, Melbourne.
June 23.

[1] Boyer, G., *Nature*, **185**, 327 (1960).
[2] Karnofsky, Ridgway and Patterson, P., *Endocrinol.*, **48**, 596 (1951).
[3] Sames and Leatham, J., *Proc. Soc. Exp. Biol. Med.*, **78**, 231 (1951).
[4] Evans, H., *Proc. Soc. Exp. Biol. Med.*, **83**, 36 (1953).

Degradation of Benzylpenicillin at *p*H 7·5 to D-Benzylpenicilloic Acid

In acid aqueous solution, benzylpenicillin (I) becomes rearranged to D-benzylpenicillic acid[1a], and in alkaline aqueous solution, it is hydrolysed to the α-diastereomer of D-benzylpenicilloic acid[1b] (II). The product of degradation of benzylpenicillin in aqueous solution at *p*H 7·5 has not been identified. During work on the mechanism of antigenicity of penicillin, it was necessary to investigate the degradation pathway of benzylpenicillin under physiological conditions. It was found that in aqueous solution at *p*H 7·5, benzylpenicillin is degraded to D-benzylpenicilloic acid, which appeared to be present as a mixture of its diastereomers.



A solution of 714 mgm. (2·0 millimols) of sodium benzylpenicillin in 100 ml. of 0·2 *M* phosphate buffer was kept at 37° C. for 120 hr. During this time, the optical rotation fell from $\alpha(D,29) = +4{\cdot}1°$ for the fresh penicillin solution to $\alpha(D,29) = +0{\cdot}85°$. (Optical rotations were measured in a Schmidt and Haensch polarimeter with a 2-decimetre cell.) Ultra-violet spectrophotometry showed only the formation of an absorption peak at λ322 mμ and a decrease in ultra-violet end absorption. The peak at λ322 mμ has been demonstrated to be due to the formation of a very small quantity of D-benzylpenicillenic acid (V) by re-arrangement of benzylpenicillin[2a]. The decrease in optical rotation and in ultra-violet end absorption is consistent with the formation of D-benzylpenicilloic acid[1c,2b]. Also consistent with the formation of D-benzylpenicilloic acid is the observation that degradation of benzylpenicillin aqueous solution at *p*H 7·5 caused the liberation of a new acid group. This was indicated by the fact that additions of alkali throughout the course of the degradation were necessary in order to maintain the *p*H at 7·5.

Treatment of the reaction solution with one molar equivalent of mercuric chloride at *p*H 2·5 caused the immediate precipitation of the mercuric mercaptide of D-penicillamine (III) and the liberation of a Schiff aldehyde-positive compound into the supernatant liquid. This aldehyde was isolated as its 2,4-dinitrophenylhydrazone derivative by Shriner and Fuson's method[3]; yield, 500 mgm. (70 per cent). After crystallization from 95 per cent ethanol, the yellow needles melted at 193–194° C. (dec.); the reported melting point for benzylpenilloaldehyde 2,4-dinitrophenylhydrazone is 194–195° C. (dec.)[2c]. A mixed melting point of this compound with synthetic benzylpenilloaldehyde 2,4-dinitrophenylhydrazone was 193–195° (dec.). (Benzylpenilloaldehyde was prepared by synthesis[2d] and converted to its 2,4-dinitrophenylhydrazone by Shriner and Fuson's method[3]; after crystallization from 95 per cent ethanol, the yellow needles melted at 194–195° C. (dec.).) These melting-point data indicate that the phenylhydrazone derivative isolated was indeed benzylpenilloaldehyde 2,4-dinitrophenylhydrazone.

Mercuric chloride in aqueous acid solution immediately degrades D-benzylpenicilloic acid to the mercuric mercaptide of D-penicillamine (III), benzylpenilloaldehyde (IV), and carbon dioxide[1d]. The isolation, by this means, of benzylpenilloaldehyde

in 70 per cent yield indicates that benzylpenicillin was hydrolysed to D-benzylpenicilloic acid.

The hydrolysis of sodium benzylpenicillin in *p*H 7·5 buffer yielded a monosodium benzylpenicilloate solution with a considerably less positive optical rotation ($\alpha(D,29) = +0{\cdot}85°$) than that of a freshly prepared equimolar buffer solution (*p*H 7·5) of monosodium D-α-benzylpenicilloate ($\alpha(D,29) = +1{\cdot}93°$). (Monosodium D-α-benzylpenicilloate was prepared by alkaline hydrolysis of sodium benzylpenicillin[2e], and recrystallized twice from water–acetone; melting point, 154–155° C. (dec.).) On standing at 37° C., the α-diastereomer underwent mutarotation; the optical rotation of this D-α-benzylpenicilloate solution fell to $\alpha(D,29) = +0{\cdot}44°$ after 60 hr. These observations suggest that the D-benzylpenicilloic acid formed by hydrolysis at *p*H 7·5 of benzylpenicillin may be present as a mixture of its diastereomers[1e].

The hydrolysis of benzylpenicillin at *p*H 7·5 may proceed through two possible pathways: (1) the α-diastereomer of D-benzylpenicilloic acid may be formed primarily by direct hydrolysis of the β-lactam linkage of benzylpenicillin, followed by epimerization to yield a mixture of its diastereomers; (2) benzylpenicillin may become degraded by slow rearrangement to D-benzylpenicillenic acid (V), which is rapidly hydrolysed to yield a mixture of diastereomers of D-benzylpenicilloic acid directly. In support of the latter pathway are the following observations: a small quantity of D-benzylpenicillenic acid was detected in the degrading benzylpenicillin buffer solution (see above). In aqueous solution at *p*H 7·5 at 37° C., D-benzylpenicillenic acid is hydrolysed rapidly to D-benzylpenicilloic acid ($t_{1/2} = 6{\cdot}5$ min.) (unpublished work). It must be noted that the degradation at *p*H 7·5 of benzylpenicillin may proceed through both these pathways simultaneously. Further work on this problem is in progress.

I thank Drs. E. Perlman, S. S. Schneierson, and H. Sobotka of the Mount Sinai Hospital for their interest during the course of this work, and the Charles Pfizer Laboratories, Brooklyn, New York, for a generous supply of sodium benzylpenicillin.

Bernard B. Levine*

Departments of Chemistry and Microbiology,
Mount Sinai Hospital,
New York, N.Y.

* Dazian Foundation Research Fellow, 1958–59.

[1] Cited by: Florey, H. W., Chain, E., Heatley, N. G., Jennings, M. A., Sanders, A. G., Abraham, E. P., and Florey, M. E., "Antibiotics", **2** (Oxf. Univ. Press, London, 1949). (*a*) p. 889; (*b*) p. 840; (*c*) p. 803; (*d*) p. 839; (*e*) see p. 844 for a discussion of the stereochemistry of penicilloic acids.

[2] Cited in: "Chemistry of Penicillin", edit. by Clarke, H. T., Johnson J. R., and Robinson, R. (Princeton Univ. Press, Princeton, N.J., 1949): (*a*) pp. 162, 431; (*b*) p. 426; (*c*) p. 477; (*d*) p. 482; (*e*) p. 573.

[3] Shriner, R. L., and Fuson, R. C., "The Systematic Identification of Organic Compounds", 171 (John Wiley and Sons, Inc., New York, 1948).

Formation of D-Penicillamine-Cysteine Mixed Disulphide by Reaction of D-Benzylpenicilloic Acid with Cystine

As part of a study of skin sensitization to benzylpenicillin, I have investigated the behaviour of D-benzylpenicilloic acid (4-carboxy-5,5′-dimethyl-α-phenylacetamido-2-thiazolidine acetic acid). This compound was found to give allergic cross-reactions of the contact dermatitis type with both benzylpenicillin and D-penicillamine (unpublished work). The allergic activity of D-penicillamine is probable due to its ability to react with cystine disulphid. linkages of epidermal proteins to form D-penicillamine cysteine mixed disulphide residues[1]; the allergic cross-reactions between D-benzylpenicilloic acid and D-penicillamine might be due to the ability of the former to react also with disulphide linkages of epidermal proteins to form D-penicillamine-cysteine residues. There are no reports in the literature on the reactivity of D-benzylpenicilloic acid with either thiols or disulphides; I report here the reaction between D-benzylpenicilloic acid and cystine in aqueous solution at *p*H 7·5.

Cystine (96 mgm.) was suspended in 0·1 *M* aqueous solution of monosodium D-α-benzylpenicilloate at *p*H 7·5. (Monosodium D-α-benzylpenicilloate was prepared by alkaline hydrolysis of benzylpenicillin[2] and recrystallized twice from water–acetone; melting point, 154–155° C. (dec.). The material was found to be free of D-penicillamine by a paper chromatographic method capable of detecting less than 0·1 (unpublished work).) This reaction mixture was stirred at 37° C. for 24 hr. A 0·1 *M* aqueous solution of monosodium D-α-benzylpenicilloate under the same conditions served as a control. Aliquots of the reaction mixture supernatant and the control solution were analysed by descending paper chromatography with phenol–water as solvent. Monosodium D-α-benzylpenicilloate, D-penicillamine, cystine, and D-penicillamine-cysteine were chromatographed simultaneously to serve as standards. (D-Penicillamine-cysteine was prepared by the method of Tabachnick *et al.*[1], melting point, 195° C. (dec.).) The results are shown in Fig. 1. The reaction mixture supernatant contained another ninhydrin-positive compound with R_F identical with that of D-penicillamine-cysteine (R_F 0·33). This compound was eluted from the chromatogram. It gave a positive nitroprusside reaction only after reduction with alkaline cyanide solution, and yielded cysteic acid

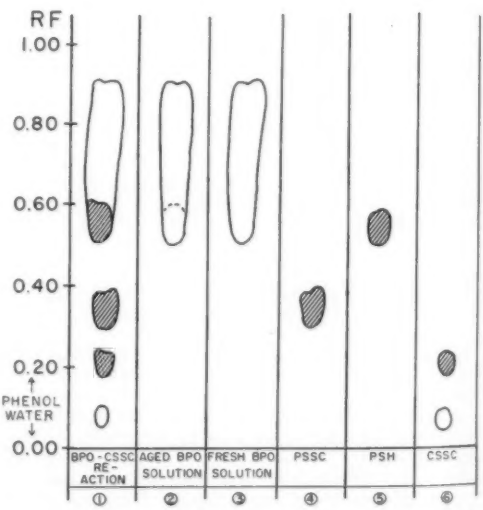


Fig. 1. Phenol–water paper chromatogram of: (1) the D-benzylpenicilloic acid (*BPO*)–cystine (*CSSC*) reaction solution; (2) the aged D-benzylpenicilloic acid (*BPO*) solution; (3) a freshly prepared D-benzylpenicilloic acid (*BPO*) solution; (4) D-penicillamine-cysteine (*PSSC*) standard; (5) D-penicillamine (*PSH*) standard; (6) cystine (*CSSC*) standard. Colour development was with ninhydrin

and penicillamine sulphonic acid after oxidation with aqueous bromine. These reactions have been described previously for D-penicillamine-cysteine[1]. These results indicate that D-penicillamine-cysteine mixed disulphide was formed by reaction of D-benzylpenicilloic acid with cystine.

The chromatogram of the reaction mixture supernatant also showed the presence of another spot superimposed on the D-benzylpenicilloic acid streak (Fig. 1). Its R_F was identical to that of D-penicillamine. Elution of this spot and oxidation with aqueous bromine yielded only penicillamine sulphonic acid. This spot appears to be due to the presence of D-penicillamine in the reaction mixture supernatant, probably formed by a secondary reaction between D-penicillamine-cysteine mixed disulphide and cysteine, the products of the D-benzylpenicilloic acid–cystine reaction. In aqueous solution at *p*H 7·5, D-penicillamine-cysteine was found to react with cysteine to yield D-penicillamine and cystine.

The formation of the mixed disulphide of D-penicillamine and glutathione was similarly observed when monosodium-D-α-benzylpenicilloate was treated with oxidized glutathione in aqueous solution at *p*H 7·5.

The mechanism of the D-benzylpenicilloic acid–cystine reaction is unknown. Early experiments suggest that the reactive form of D-benzylpenicilloic acid may be D-benzylpenamaldic acid (α-phenylacetamido-β-(α′-amino-β′-mercapto*iso*valeric acid) acrylic acid), traces of which appear to exist in tautometric equilibrium with D-benzylpenicilloic acid.

D-Benzylpenicilloic acid may react also with cystine disulphide linkages of epidermal proteins to form D-penicillamine-cysteine mixed disulphide residues. This chemical reaction might explain the allergic cross-reactivity between D-benzylpenicilloic acid and D-penicillamine; both of which may react with cystine disulphide linkages of epidermal proteins to form the D-penicillamine-cysteine antigenic-determinant group. Since benzylpenicillin is hydrolysed to D-benzylpenicilloic acid under physiological conditions[2], its allergic cross-reactivity with the latter and D-penicillamine (unpublished work) may be similarly explained.

I wish to thank Drs. E. Perlman, S. Schneierson and H. Sobotka of the Mount Sinai Hospital for their interest during the course of this work.

BERNARD B. LEVINE*
Departments of Chemistry and Microbiology,
Mount Sinai Hospital,
New York, N.Y.

* Dazian Foundation Research Fellow, 1958–59.

[1] Tabachnick, M., Eisen, H. N., and Levine, B. B., *Nature*, **174**, 701 (1954).
[2] Cited in: "Chemistry of Penicillin", 573 (edit. by Clarke, G. T., Johnson, J. R., and Robinson, R. (Princeton Univ. Press, Princeton, N.J., 1948).
[3] Levine, B. B., *Nature*, see preceding communication.

New Inhibitor of Monoamine Oxidase

THE following is the initial report of a structurally unique monoamine oxidase inhibitor, N-benzyl-N-methyl-2-propynylamine hydrochloride (*A*19120). This work includes *in vitro* action of this inhibitor on rat liver mitochondria as well as *in vivo* inhibition of monoamine oxidase of mouse brain and the liver.

The mitochondria were isolated from homogenate of rat liver by the differential centrifugation method of Hogeboom *et al.*[1], and suspended in *M*/15 phosphate buffer, *p*H 7·2. Homogenates of whole brain and liver tissue from test or control male mice were prepared by adding 1 ml. of the phosphate buffer for each 0·2 gm. tissue and homogenizing with Potter–Elvehjem homogenizers.

The monoamine oxidase activity of mitochondria or tissue homogenates was measured by a Warburg manometric technique at 37° C. The flasks contained tissue or mitochondria equivalent to 0·2 gm. of original tissue, *M*/15 phosphate buffer *p*H 7·2, and (in specified experiments) inhibitor. Serotonin creatinine sulphate was added at zero time to give a final concentration of *M*/100 in the 2·0-ml. reaction mixture. During the pre-incubation–equilibration period reaction mixtures were oxygenated for 5–10 min. Calculations were based on the uptake of oxygen during the first 60 min. of reaction-time.

The *in vitro* activity of N-benzyl-N-methyl-2-propynylamine hydrochloride was compared with that of several known compounds. All were pre-incubated for 25 min. The concentration of drug which produces 50 per cent inhibition of mitochondrial monoamine oxidase was read off the straight-line graph obtained by plotting the log concentration of inhibitor against per cent inhibition. The results were: *A*19120, 9×10^{-7} *M*; nialamide, 9×10^{-7} *M*; harmaline, 1×10^{-6} *M*; β-phenyl*iso*propyl hydrazine, 4×10^{-6} *M*; and iproniazid, 7×10^{-6} *M*.

Table 1. EFFECT OF PRE-INCUBATION TIME ON INHIBITION OF MONOAMINE OXIDASE *A*19120, HARMALINE AND IPRONIAZID

Inhibitor	Concentration *M*/1	Per cent inhibition after pre-incubation time* 0 min.	8 min.	15 min.	30 min.
*A*19120	5×10^{-7}	12	19	19	40
	1×10^{-6}	15	27	37	61
	2×10^{-6}	21	41	56	83
Iproniazid	$7{\cdot}5 \times 10^{-6}$	4	12	25	76
Harmaline	5×10^{-7}	39	—	37	40

* Values as per cent of controls.

Table 1 summarizes the effect of increasing the pre-incubation time at 37° C. with *A*19120, iproniazid, and harmaline. Both *A*19120 and iproniazid show progressive inhibition with increasing pre-incubation time whereas harmaline does not.

In another series of experiments substrate was added to the mitochondria and the uptake of oxygen followed for 30 min., then the inhibitor was added from a second side-arm at a concentration which would have given 50 per cent inhibition of monoamine oxidase with 25 min. pre-incubation. Compound *A*19120 depressed the rate of oxygen consumption 22 per cent over controls, harmaline 54 per cent and iproniazid had no effect. This indicates that *A*19120 and iproniazid may not be identical in their mechanism of inhibition of monoamine oxidase.

Determinations for ammonia production by the Conway microdiffusion technique[2] and serotonin disappearance, by the method of Udenfriend *et al.*[3], were carried out on samples from Warburg vessels 60 min. after the addition of substrate to mitochondria. The ratios of oxygen/ammonia, oxygen/serotonin and ammonia/serotonin were 0·8–1·3 for controls, for *A*19120 at 1×10^{-6} *M*, and for iproniazid at $1{\cdot}25 \times 10^{-6}$ *M*. This agreement between the theoretical ratio of 1·0 and experimental values indicates that these inhibitors probably act directly on the monoamine oxidase–serotonin system.

The reversibility of the inhibition effect was studied by a procedure similar to that of Udenfriend *et al.*[4]

Table 2. Duration of Action of Inhibition of Monoamine Oxidase in Mice by A19120 or Iproniazid. Dose, 200 mgm./kgm.

Days	Per cent inhibition			
	A19120		Iproniazid	
	Brain	Liver	Brain	Liver
1/6	68	73		
1	73	93	78	85
2	78	74		
3	74	65		
4	69	59		
5	59	49	58	37
6	55	44		
7	44	34		
8	50	38		
9	48	33		
10	17	6	30	12
12	17	0		
14	17	7	21	9

except that the activity of monoamine oxidase was measured by the Warburg technique. The inhibition produced by *A*19120 at 3×10^{-6} *M*, or iproniazid at 1×10^{-5} *M* (30 min. pre-incubation), was not reversed with four washings, whereas inhibition by 3×10^{-6} *M* harmaline was decreased from 84 to 50 per cent.

In a single experiment the duration of inhibition of monoamine oxidase of mouse brain and liver produced by one oral dose of 200 mgm./kgm. of *A*19120 or the same dose of iproniazid was determined. The results are in Table 2. Significant inhibition of brain monoamine oxidase occurred for at least seven days after administration of *A*19120. Although liver inhibition is less than that of brain after the first day, there is probably no preferential inhibition of the brain monoamine oxidase by *A*19120 at this pharmacologically high dose. Preliminary data from our laboratories also indicate that *A*19120 causes maximal increase in brain norepinephrine. Pharmacological work is now in progress and will be reported in a separate publication.

The results obtained in this work show that *A*19120 is a potent inhibitor of monoamine oxidase of a new structural type, and thus the present concepts of inhibition of monoamine oxidase should be enlarged.

J. D. Taylor
A. A. Wykes
Y. C. Gladish
W. B. Martin

Abbott Laboratories,
North Chicago, Illinois.

[1] Hogeboom, G. H., Schneider, W. C., and Palade, G. E., *J. Biol. Chem.*, 172, 619 (1948).
[2] Conway, E. J., "Microdiffusion Analysis and Volumetric Error" (Macmillan Co., N.Y., 1958).
[3] Udenfriend, S., Weissbach, H., and Clark, C. T., *J. Biol. Chem.*, 215, 337 (1955).
[4] Udenfriend, S., Witkop, B., Redfield, B. G., and Weissbach, H., *Biochem. Pharmacol.*, 1, 160 (1958).

Syntheses of Glucose, Cellobiose and Other Saccharides by *Acetobacter acetigenum* in a Lactate-buffered Glycerol Medium

We have cultivated *Acetobacter acetigenum* N.C.I.B. 8132, a cellulose-forming micro-organism, in 10 litres of medium in 40 'Glaxo' flasks, which were shaken during incubation at 30° for twenty-one days. The medium contained (gm./l.): potassium dihydrogen phosphate, 2·0; crystalline magnesium sulphate, 1·0; crystalline ferrous sulphate, 0·01; ammonium hydrogen phosphate, 2·5; calcium D-pantothenate, 0·002; riboflavin, 0·002; biotin, 0·0001; glycerol, 30; lactic acid, 10·2; the *p*H value was adjusted with potassium hydroxide to 4·6. The metabolism solution was Seitz-filtered to remove cells and cellulose and concentrated to 500 ml. over barium carbonate, *in vacuo* at 35°. Activated charcoal, 'Ultrasorb *S.C.* 120/240' (British Carbo Norit Union) as used by Hughes and Whelan[1], was sieved through a British Standards 170 mesh and the material retained on the sieve was used to pack two 'Pyrex' glass columns (138 × 7·8 cm.) to a depth of 105 cm. In each column 1·9 kgm. of this charcoal rested on a pad of glass-wool overlayed with 100 gm. of 'Celite' added as a slurry in water. The columns were washed each with 5 litres of 50 per cent (v/v) aqueous ethanol and then each with 20 litres of distilled water. After removal of insoluble barium salts the metabolism concentrate was rediluted to 10 litres with distilled water and passed through the columns during 14 hr. Each column was eluted with 80 litres of distilled water followed by aqueous ethanol solutions (each of 50 litres) in which the respective ethanol percentages (v/v) were 0·5, 5, 10, 20, 35, and 50. Six aqueous ethanolic fractions, A_1–A_6, were collected and concentrated at 35°. Portions of each were de-ionized with 'Zeo-Karb 225' and 'Deacidite *E*' and chromatographed on paper against authentic sugars using (*a*) *n*-propanol/water/ethylacetate, 7:2:1 by volume; (*b*) pyridine/water/ethylacetate, 1:2:2 by volume; (*c*) ethyl acetate/acetic acid/water, 3:1:3 by volume. The chromatograms gave evidence of the presence of the following sugars: A_2, glucose, fructose, xylose, and ribulose; A_3, similar to A_2 together with a trace of cellobiose; A_4, mainly cellobiose; A_5, cellotriose and cellotetraose. Fraction A_3 was then de-ionized and submitted to column partition chromatography according to the procedure of Lemieux, Bishop and Pelletier[2], which involves the use of 'Celite No. 535' to hold the stationary phase. After irrigation with water-saturated *n*-butanol, the column was extruded and cut into zones as determined by an alkaline permanganate spray. A fraction was separated which, after several further passages through the 'Celite' and a final purification by band paper chromatography, yielded 45 mgm. of a substance which had the mobility of glucose when chromatographed in two different solvent systems. An acetate was prepared by heating with anhydrous sodium acetate and acetic anhydride, and after purification this had m.p. 131°, alone or mixed with authentic β-penta-acetyl glucose of m.p. 131°. The infra-red spectra of both acetates were compared and no significant differences could be observed. (Found: C, 49·3; H, 5·8 per cent. Calc. for $C_{16}H_{22}O_{11}$: C, 49·2; H, 5·7 per cent.)

The procedures by which glucose had been isolated were now applied to fraction A_4, yielding 52 mgm. of a substance which showed the mobility of cellobiose when run on paper chromatograms in different solvent systems. The substance was acetylated with pyridine and acetic anhydride and the purified derivative had m.p. 191°, unchanged by admixture with authentic β-cellobiose octa-acetate, m.p. 191°. (Found: C, 49·7; H, 5·7 per cent. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6 per cent.) The infra-red spectra of this octa-acetate and that of authentic β-cellobiose octa-acetate were without significant differences.

When lactate is metabolized by *A. acetigenum* in absence of other sources of carbon it is mainly oxidized through acetate to carbon dioxide, though in certain liquid media it can serve as a source for formation of cellulose[3]. It seems probable that in our experiments the lactate played a dual part, some of it suffering oxidation and thereby sparing glycerol for cellulose synthesis, and some of it undergoing conversion to a metabolite on the pathway to cellulose. So far as we are aware, neither glucose nor cellobiose has hitherto

been isolated from among the products of bacterial action on either glycerol or lactate, although the formation of glucose in cultures of *Poria vaillantii* on a glycerol medium has been reported by Sison and Schubert[4], while Horecker[5] has suggested a cyclic mechanism by which this conversion may be effected.

It is proposed to report the work in further detail elsewhere.

One of us (K. R.) acknowledges the receipt of an Imperial Chemical Industries Research Fellowship.

C. P. JACKSON
K. RAMAMURTI

College of Science and Technology,
University of Manchester.

[1] Hughes, R. C., and Whelan, W. J., *Chem. and Indust.*, 884 (1958).
[2] Lemieux, R. U., Bishop, C. T., and Pelletier, G. E., *Can. J. Chem.*, **34**, 1365 (1956).
[3] Bourne, E. J., and Weigel, H., *Chem. and Indust.*, 132 (1954).
[4] Sison, B. C., and Schubert, W. J., *Nature*, **181**, 1015 (1958).
[5] Horecker, B. L., *Brewers Digest*, **28**, 214 (1953).

Distribution of Protein-bound Aminoazo Dyes in the Rat Liver Microsome

EVIDENCE has been obtained that rat liver microsomes are the site where the formation of protein-bound aminoazo dyes occurs[1–3]. On the other hand, one of the most important parts played by the microsome is known to be protein synthesis. Littlefield *et al.*[4] further fractionated the microsome by means of sodium deoxycholate and pointed out that the protein in the deoxycholate-insoluble fraction, ribonucleoprotein particles, showed a particularly high turn-over rate, suggesting that protein is in fact synthesized in this fraction. This suggestion was supported by other authors[5–7].

In investigating the carcinogenesis due to aminoazo dyes, it is an inevitable question whether the protein-bound dye is detectable even in the fraction with such a high turn-over rate as the microsome ribonucleoprotein fraction. Despite the fact that Arcos and Arcos[3] are inclined to the view that no protein-bound dye was found in this fraction, we repeated a similar experiment but on a somewhat larger scale, since their evidence seemed to be inconclusive.

Rats which were fed 3′-methyl-4-dimethylaminoazobenzene coated on rice (0·06 per cent) were killed after 2 weeks when the protein-bound dye is considered to be a maximum. The liver microsomes were prepared according to Schneider's method[8] and washed once with 0·25 *M* sucrose solution. The unsedimented fluid was designated as 'supernatant fraction'. The microsomes were further fractionated by means of sodium deoxycholate as proposed by Littlefield *et al.*[4]. Thus we obtained three fractions, namely, deoxycholate-insoluble fraction (ribonucleoprotein fraction) and deoxycholate-soluble fraction from microsomes and the supernatant fraction. A small portion of each fraction was withheld for the protein[9] and ribonucleic acid[4] assays and the rest was subjected to protein-bound dye (polar dye) determination[10,11].

The results are summarized in Table 1. This clearly shows that a definite amount of protein-bound dye is detected in the ribonucleoprotein fraction. The total amount of this protein-bound dye in the ribonucleoprotein fraction is obviously lower than that in the other fractions. If, however, the protein-bound dye concentration is expressed on a per unit mgm. protein basis, the figures for the three fractions stand very closely to each other.

Table 1. DISTRIBUTION OF POLAR DYE AMONG CELL FRACTION

	Fraction	Microsome deoxycholate -insoluble	Microsome deoxycholate -soluble	Supernatant
Exp. 1	Liver used (gm.)	87		
	Protein (mgm.)	105	750	
	Ribonucleic acid (mgm.)	63	3·0	
	Polar dye (γ)*	2·6	27	
	Polar dye (γ)/100 mgm. protein	2·5	3·6	
Exp. 2	Liver used (gm.)	60		
	Protein (mgm.)	65	530	2,700
	Ribonucleic acid (mgm.)	56	18	
	Polar dye (γ)*	2·0	15	93
	Polar dye (γ)/100 mgm. protein	3·1	2·8	3·4

* The amount of polar dye is expressed in terms of DAB tentatively.

The significance of the finding must await further experiments, which are now being carried out here.

This experiment was supported partly by the grant from the Ministry of Education, Japan.

T. YAMADA
M. MATSUMOTO
S. KANDA
H. TERAYAMA

Department of Biophysics and Biochemistry,
Faculty of Science,
University of Tokyo,
Tokyo.

[1] Hultin, T., *Exp. Cell Res.*, **10**, 71, 697 (1956); **13**, 47 (1957).
[2] Gelboin, H. W., Miller, J. A., and Miller, E. C., *Cancer Res.*, **18**, 618 (1958).
[3] Arcos, J. C., and Arcos, M., *Biochim. Biophys. Acta*, **28**, 9 (1958).
[4] Littlefield, J. W., Keller, E. B., Gross, J., and Zamecnik, P. C., *J. Biol. Chem.*, **217**, 111 (1955).
[5] Alfrey, V. G., Daly, M. M., and Mirsky, A. E., *J. Gen. Physiol.*, **37**, 157 (1953).
[6] Hultin, T., *Exp. Cell. Res.*, Supp., **3**, 210 (1955).
[7] Simkin, J. L., and Work, T. S., *Biochem. J.*, **65**, 307 (1957).
[8] Schneider, W. C., and Hogeboom, G. H., *J. Biol. Chem.*, **183**, 123 (1950).
[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
[10] Terayama, H., Kusama, K., Teruya, K., Kuroda, S., and Nakayama, T., *Gann*, **49**, 85 (1958).
[11] Terayama, H., Kusama, K., and Aoki, T., *Gann*, **49**, 97 (1958).

Water-soluble, Non-fibrous Deoxyribonucleoprotein from Calf Thymus Nuclei

A WATER-SOLUBLE, non-viscous form of deoxyribonucleoprotein has been isolated from purified calf thymus nuclei by a process that minimizes enzyme degradation. Previously the only preparation of deoxyribonucleoprotein generally accepted as undegraded has been the fibrous one, forming gels in water and only slightly soluble, with a molecular weight of 17 million[1].

Fresh calf thymus glands are frozen immediately on removal from the animal and ground to a powder in the frozen state in the presence of 'dry ice'. Only this procedure has been found to produce intact, round nuclei, as confirmed by microscopic examination, without excessive clumping, when the ground material is suspended in the purification medium at 0–4° C.

The purification medium is modified from those of Dounce *et al.*[2] and Philpot and Stanier[3], and is composed of 0·44 *M* sucrose, 40 per cent glycerol, 0·039 *M* sodium glycerophosphate, 0·019 *M* citrate buffer (*p*H 6·1, ionic strength 0·15).

Table 1. Molecular Weights and Parameters for Average-sized Particles of Fibrous and Non-fibrous Deoxyribonucleoprotein

Prep.	Solvent	ν_0 (c.c./c.c.)†	a/b	Av. $S(20w) \times 10^{13}$	$M \times 10^{-6}$
A. Fibrous*	1 *M* NaCl-PO_4	4,030±770	291±31‡	43±1·5§	15·9±2·4
B. Non-fibrous, stored at 4°	1 *M* NaCl-PO_4	1,002	135	8·2	0·75
Non-fibrous, lyophilized	1 *M* NaCl-PO_4	674	108	9·5	0·79
Same	0·7 *mM* PO_4	591	96	7·3	0·49

* This preparation, entirely corresponding, was made and studied at Pollards Wood Research Station, Chalfont St. Giles, Bucks., England.
† This viscosity increment was obtained from the intrinsic viscosity at zero gradient, assuming a partial specific volume of 0·658 (ref. 8).
‡ Limits of error calculated to take into account the effect on viscosity of the known heterogeneity of the material.
§ The error is the standard deviation for the scattering of points from the straight line calculated by least mean squares obtained for the line portion of a typical sedimentation coefficient curve.

The intact nuclei are rapidly washed with this medium and centrifuged at 1,200*g*. The process is repeated four times, followed by a slow-speed centrifugation to remove whole cells and clumps. Immediately afterwards, the nuclei are extracted with water and centrifuged three times at 4° C. This washing with water appears to be of special significance for the chemical interactions determining the state of the deoxyribonucleoprotein, and will be the subject of a forthcoming publication. The swollen gel of nuclei is adjusted to 0·14 *M* sodium chloride and hand-homogenized mildly but rapidly, the process taking only about a minute. A finely divided mass of disrupted nuclei is thus obtained, giving a solution of non-fibrous deoxyribonucleoprotein after dialysis against 0·7 m*M* phosphate at *p*H 9, at 4° C. and, finally, clarification at 25,000*g*. The resulting solution was used for the viscosity and sedimentation work reported here.

It should be pointed out that fibrous deoxyribonucleoprotein is obtained from the nuclei washed in the medium, if, instead of the washing with water and hand homogenization in 0·14 *M* sodium chloride, the purified nuclei are dissolved directly in 1 *M* sodium chloride and clarified by centrifugation. The fibrous deoxyribonucleoprotein thus obtained and described here corresponds to preparations previously reported in the literature[1,4].

Viscosities were measured in 4-bulbed Ubbelohde viscometers, in those of the Frampton type[5], and in a Couette viscometer, to give a range of velocity gradients of 1–600 sec.$^{-1}$.

The distribution of sedimentation coefficients corrected to 20° C. in water was determined in the Spinco Model *E* analytical ultracentrifuge and calculated by the method of Shooter and Butler[6]. The solvent used was 1 *M* sodium chloride, 0·7 m*M* phosphate, *p*H 7·1 and 0·7 m*M* phosphate for the non-fibrous deoxyribonucleoprotein only. Ultraviolet absorption optics permitted measurements at concentrations as low as 0·01 per cent (optical density equal to 1) or less.

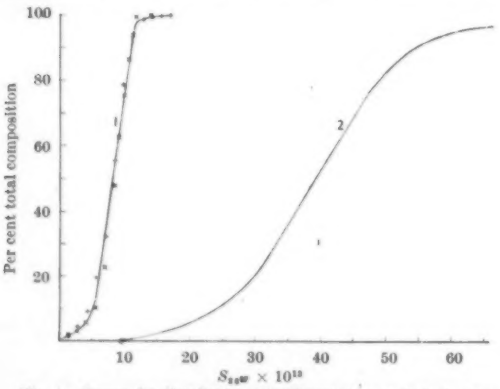


Fig. 1. Curves for distribution of sedimentation coefficients, corrected to water at 20° C., for non-fibrous and fibrous deoxyribonucleoprotein in 1 *M* sodium chlorate, 0·7 m*M* phosphate. Curve 1: Non-fibrous deoxyribonucleoprotein at a concentration of 0·091 mgm./c.c. Pluses—at 11·95 minutes after the extrapolated zero time (6); crosses—at 23·95 minutes after zero time. Curve 2: Fibrous deoxyribonucleoprotein from unextracted nuclei at 0·037 mgm./c.c. (courtesy of Dr. K. V. Shooter)

Concentrations were determined by nitrogen analysis and dry weight (indicating 17 per cent nitrogen in deoxyribonucleoprotein), or by the ultraviolet absorption at 260 mμ for preparations for which the extinction coefficients were known.

Viscosity and sedimentation determinations for both non-fibrous and fibrous deoxyribonucleoprotein, summarized in Table 1, show the striking contrast between these two forms. Fig. 1 shows that not only is the average sedimentation constant of the non-fibrous deoxyribonucleoprotein much lower than that of the fibrous, but also that it is distinctly more homogeneous; thus there is an increase in proportionate slope and also the last 5 per cent of highly aggregated material is eliminated for the non-fibrous sample. Whereas the fibrous deoxyribonucleoprotein shows an average sedimentation constant of 39 *S* at a concentration of 0·037 mgm./c.c. in molar salt, that observed for the non-fibrous preparation is strikingly lower, namely, 8·2 *S*. Paralleling this contrast is that observed for the axial ratio a/b for the fibrous form being 291 and that for the non-fibrous form 135 in molar salt or 96 in dilute buffer. Molecular weight calculations from these data lead to the values of 16 million for fibrous deoxyribonucleoprotein and 0·77 million for the non-fibrous form in molar salt for the average-sized particles, assuming effective hydrodynamic ellipsoids[7].

All the results clearly support the hypothesis that there are much smaller sub-unit particles of deoxyribonucleoprotein than those of fibrous deoxyribonucleoprotein, commonly accepted as such. Enzymatic degradation during the preparative steps is most unlikely; first, because the mild hand homogenization, the all-important step connecting fibrous preparations with the non-fibrous ones, is accomplished so rapidly, and secondly, because in a control experiment, nuclei washed with the purification medium only yielded fibrous deoxyribonucleoprotein after standing at 4° for as long as 4 days in 0·14 *M* sodium chloride.

Work now in progress indicates that the deoxyribonucleic acid obtained from the non-fibrous deoxyribonucleoprotein by salt and detergent dissociation has an average sedimentation coefficient of 5·3 *S* in molar salt, which is lower, as expected, than for the corresponding deoxyribonucleoprotein preparation. This finding lends further support to the view that the non-fibrous preparations are comprised of particles

which are sub-units of the conventional fibrous particles.

This work was supported in part by a grant, No. A 2401, of the National Institutes of Health. I thank Prof. J. A. V. Butler for his hospitality, and Dr. K. V. Shooter for his collaboration and helpful advice.

RICHARD S. WELSH*
Chemistry Department, University of Redlands, Redlands, California.

* Advanced Research Fellow of the American Heart Association.

[1] Doty, P., and Zubay, G., *J. Molecular Biol.*, **1**, 1 (1959).
[2] Dounce, A. L., Witter, R. F., Monty, K. J., Pate, S., and Cottone, M. S., *J. Biophys. and Biochem. Cytol.*, **1**, 139 (1955).
[3] Philpot, J. St. L., and Stanier, J. E., *Biochem. J.*, **63**, 214 (1956).
[4] Mirsky, A. E., and Pollister, A. W., *J. Gen. Physiol.*, **30**, 101 (1946). Frick, G., "Studies on Desoxyribonucleoprotein and Desoxyribonucleic Acid," dissertation, Uppsala (1954).
[5] Frampton, V. C., *J. Biol. Chem.*, **129**, 223 (1939).
[6] Shooter, K. V., and Butler, J. A. V., *Trans. Farad. Soc.*, **52**, 734 (1956).
[7] Scheraga, H. A. and Mandelkern, L., *J. Amer. Chem. Soc.*, **71**, 536 (1949).
[8] Carter, A. C., *J. Amer. Chem. Soc.*, **63**, 1960 (1941).

PHYSIOLOGY

Adenosine Triphosphate and Maintenance of Shape of the Human Red Cells

ALTHOUGH the mechanism with which red blood cells maintain their biconcave shape has been studied by a number of investigators, it has not yet been clarified. Some twenty years ago Furchgott and Ponder[1] proposed the existence of an antisphering factor in the blood plasma, but later Ponder[2] himself cast doubt on this. More recently, Prankerd[3] suggested that the red cell envelope might possess certain contractile properties dependent upon a continuous regeneration of adenosine triphosphate, according to the evidence that incorporation of phosphorus-32 is low in the esters of the stroma obtained from those species with spheroidal red cells or from patients with hereditary spherocytosis. However, no detailed experiment on the shape of the cells has been reported by Prankerd or by others.

In our experiment human red cells obtained by venipuncture were washed three times with physiological saline, and a 7 per cent suspension of these cells in physiological saline was incubated at 37° C. with a final concentration of 2×10^{-2} *M* sodium fluoride. During the incubation the discoidal shape of the cells changed to a crenate one at first, and after 6 hr. almost all the cells were transformed to smooth spheres. At this time the cells were washed with cold physiological saline four times in order to wash out the fluoride thoroughly and incubated further with 5×10^{-3} *M* glucose, 1×10^{-2} *M* inosine, and 2×10^{-3} *M* adenine sulphate to restore the content of adenosine triphosphate of the cells as in the previous experiments[4]. After 2 hr. the shape of the incubated cells turned to a biconcave disk or a shallow cup-form again. The disk–sphere transformation and vice versa could be repeated several times, and the phenomenon was also observed in red cells obtained from fresh or long-preserved human blood or from rat or pig blood as well.

In parallel with the morphological observation described above, the adenosine triphosphate in the cells was estimated as acid-soluble total phosphate adsorbed on and eluted from 'Norit *A*', by Fiske and Subbarow's[5] method, or the sum of adenosine triphosphate and adenosine diphosphate content was calculated from optical density at 260 mμ after separation by means of a slightly modified Cohn and Carter's ion exchange chromatography[6]. The results shown in Table 1 indicate that more than two-thirds of the original values of nucleotides was necessary for the maintenance of discoid shape, and that the cells change to smooth spheres as the level of nucleotides decreases to one-fourth or one-fifth of the original level. As it is well established that most of the nucleotides in the blood cells belong to adenosine phosphates, especially to adenosine triphosphate[7], the amount of nucleotide phosphates estimated as above approximately equals the amount of adenosine triphosphate.

The same transformation was also observed during the preservation of blood in ACD medium at 4° C.: the biconcave disk shape changed to crenated disk at

Table 1. SHAPE OF ERYTHROCYTES AND THEIR NUCLEOTIDE CONTENT. WASHED ERYTHROCYTES; INCUBATION TEMPERATURE 37° C.; HÆMATOCRIT ABOUT 7 PER CENT

Incubation time	2 hr.			4 hr.			6 hr.		
Conditions	Nucleotide P μ*M*/100 ml.	Nucleotide μ*M*/100 ml.*	Shape	Nucleotide P μ*M*/100 ml.	Nucleotide μ*M*/100 ml.*	Shape	Nucleotide P μ*M*/100 ml.	Nucleotide μ*M*/100 ml.*	Shape
Glucose 5×10^{-3} *M*	19·2		Biconcave disk	16·5		Biconcave disk	15·2		Biconcave disk
No addition	14·6		Biconcave disk	13·6		Biconcave disk, and crenated disk	12·4		Crenated disk
Sodium fluoride 2×10^{-2} *M*	12·4		Crenated sphere	9·5		Crenated sphere, and smooth sphere	5·7		Mostly smooth sphere
Reincubation after treatment with sodium fluoride at 37° C. for 6 hr.									
Reincubation time	1 hr.			2 hr.			3 hr.		
No addition	3		Crenated sphere, and smooth sphere	3		Crenated sphere, and smooth sphere	3		Crenated sphere, and smooth sphere
Glucose 5×10^{-3} *M* Adenine 2×10^{-3} *M* Inosine 1×10^{-2} *M*		6·5	Crenated sphere, and biconcave disk		7·4	Crenated disk, and biconcave disk		7·3	Biconcave disk

* Adenosine tri- and di-phosphate μ*M*/100 ml. calculated from optical density at 260 mμ after separation by ion-exchange chromatography.

Table 2. Shape of Erythrocytes and their Adenosine Triphosphate Content during Preservation at 4° C. in ACD Medium

Period of storage	1 day	12 days	28 days	56 days	100 days
Shape of erythrocyte	Biconcave disk	Biconcave disk, crenated disk	Crenated sphere, and smooth sphere	Smooth sphere	Smooth sphere
Adenosine triphosphate μM/100 ml. cells	84	30	18	4·4	—
Adenosine triphosphate and adenosine diphosphate μM/100 ml. cells	98	70	34	5·1	—

first (after about two weeks), and then to crenated spheres and finally to smooth spheres (after more than about five weeks). Calculating from the results which were obtained by the gradient elution technique of ion-exchange column chromatography on 'Dowex 1' (formate system)[8], the adenosine triphosphate content of the cells is summed up again in Table 2 together with morphological observations. M. Nakao *et al.*[4] have reported recently that human red cells, which were spherical after storage for several weeks at 4° C. in ACD solution, transformed to a shallow bowl form after incubation with added adenine and inosine, being accompanied by a marked rise of adenosine triphosphate content in the cells.

According to earlier work[9], round ghost cells obtained from long-stored red cells changed to a goblet-like shape with the addition of 0·003–0·04 *M* or higher concentration of adenosine triphosphate (optimal *p*H 8), and they returned to the former shape upon addition of ethylenediamine tetraacetate in 0·004 *M* concentration shortly after the addition of adenosine triphosphate.

The observations presented here lead us to the following conclusions:

(1) The presence of blood plasma is not necessary for the red cells to maintain their discoidal shape.

(2) The disk–sphere transformation of red cells and vice versa can take place without any change in hydrogen ion and cation concentration and ionic strength in the medium.

(3) If adenosine triphosphate or adenine nucleotide content in red cells is greater than a certain level, the normal shape of the cells is maintained, and if it is below this level, the shape becomes crenate, and when it decreases further, below another lower level, the shape of the cells changes to a perfect sphere.

It remains obscure, however, whether the maintenance of the shape of red cells depends solely on the level of adenosine triphosphate or on the turn-over of adenosine triphosphate.

An experiment aimed at extracting actomyosin-like protein from red cell ghosts is now in progress.

Makoto Nakao
Toshiko Nakao
Saburo Yamazoe

Department of Biochemistry,
School of Medicine,
Gunma University,
Maebashi, Japan.

1 Furchgott, R. F., and Ponder, E., *J. Exp. Biol.*, **17**, 117 (1940).
2 Ponder, E., *Blood*, **9**, 227 (1954).
3 Prankerd, T. A. J., *Biochem. J.*, **58**, 622 (1954).
4 Nakao, M., Nakao, T., Tatibana, M., Yoshikawa, H., and Abe, T., *Biochim. Biophys. Acta*, **32**, 564 (1959); *J. Biochem.* (Japan).
5 Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, **66**, 375 (1925).
6 Cohn, W. E., and Carter, C. E., *J. Amer. Chem. Soc.*, **72**, 4273 (1950).
7 Bishop, C., Rankine, D. M., and Talbott, J. H., *J. Biol. Chem.*, **234**, 1223 (1959).
8 Tatibana, T., Nakao, M., and Yoshikawa, H., *J. Biochem.* (Japan), **31**, 679 (1959).
9 Nakao, M., Nakao, T., Tatibana, M., and Yoshikawa, H., *J. Biochem.* (Japan), **47**, 694 (1960).

An Abnormal Serum Protein in Experimental Poisoning by Cadmium and other Metals

It has been shown by Friberg[1] that men exposed for many years to the hazard of cadmium inhalation can excrete a protein having a low molecular weight in their urine. In order to investigate this phenomenon further, an investigation has been carried out on the changes in serum protein produced in the albino rat, following the administration of cadmium salts, using the starch-gel electrophoresis method of Smithies[2], together with the modification in the buffer system suggested by Poulik[3].

Among several other changes, it has been observed that following the intraperitoneal or subcutaneous injection of 1 mgm. per kgm. of cadmium as cadmium chloride, an abnormal protein appeared in the serum, which could be seen when the serum was analysed on

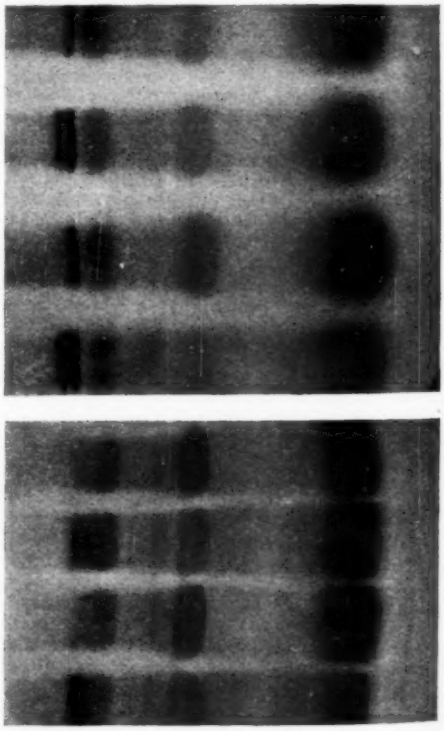


Fig. 1. Starch-gel electrophoresis of sera from albino rats, injected intraperitoneally with *a*, saline, *b*, cadmium, *c*, zinc and *d*, mercury, 1 mgm. per kgm. (as chloride) in each case. Top, continuous *tris*-citrate buffer system, 500 V., 10 m.amp., 2 V. cm.$^{-1}$ for 20 hr. Bottom, discontinuous borate, *tris*-citrate system, 500 V., 20 m.amp., 6 V. cm.$^{-1}$ for 4 hr.

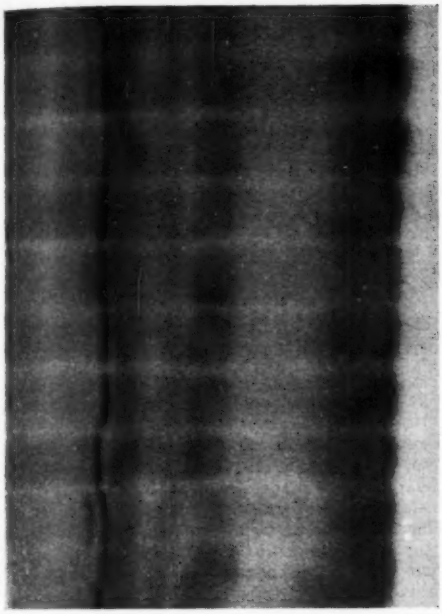


Fig. 2. Starch-gel electrophoresis of rat sera (continuous *tris*-citrate buffer system 500 V., 10 m.amp., 2 V. cm.$^{-1}$ for 20 hr.) from rats injected with 1 mgm. per kgm. of the following metals: *a*, beryllium, *b*, cadmium, *c*, chromium, *d*, cobalt, *e*, copper, *f*, iron, *g*, lead, *h*, manganese, *j*, mercury, *k*, nickel, *N*, normal saline

a Connaught starch gel, as described by Smithies. Poulik described a discontinuous buffer system for use in starch-gel electrophoresis, and it has been found that an improved picture of this abnormal protein can be obtained by using the *tris*-citrate buffer, described by him, as a continuous buffer throughout the system.

The protein, following a single injection, usually appeared within 24 hr., and it slowly disappeared in about a week. Mercury was found to behave in a similar way, when administered in the same concentration, but zinc did not (Fig. 1). At a concentration of 20 mgm. per kgm., however, zinc gave rise to the abnormal protein.

This abnormal serum protein has been isolated from the serum of cadmium-poisoned rats as follows. Preliminary experiments had established that the protein was precipitated from serum by 37 per cent saturation by ammonium sulphate, and that it moved as an α-1-globulin when submitted to paper electrophoresis or to a cellulose electrophoretic column using phosphate borate buffer as described by Gedin and Porath[4]. Forty rats were given 1 mgm. per kgm. of cadmium chloride and after 24 hr. the blood was removed by cardiopuncture. After the blood had clotted, the serum was removed and saturated ammonium sulphate solution was added to give 40 per cent saturation. The mixture was left at 0° C. for 1 hr.; the precipitated protein was centrifuged off, dissolved in distilled water, dialysed free of ammonium sulphate, and finally freeze-dried. The resultant solid was dissolved in physiological rat saline, introduced into a Porath column 2 × 150 cm. and run for 48 hr., at 1,400 V. and a current of 24 m.amp., using the phosphate–borate buffer as described. The fractions from this column were dialysed and freeze-dried, and each fraction was analysed by starch-gel electrophoresis using the continuous *tris*-citrate buffer system. The appropriate fractions were combined, redissolved and freeze-dried. 0·6 gm. of the protein was isolated. As the cadmium chloride used in the experiment had been prepared from radioactive cadmium-115, radioactivity measurements showed that the protein did not bear any cadmium.

A group of other common metals, some generally considered toxic, and others present in trace amounts in the body, was administered, all at a dose-rate of 1 mgm. per kgm. body-weight. The response of the animals (so far as the production of the protein after 24 hr. was concerned) was in the following order: mercury > beryllium, cadmium, copper > manganese > chromium, nickel. Cobalt, lead and iron did not give a visible band under these conditions (Fig. 2).

The observation that mercury and cadmium salts produce the abnormal line, in the serum of rats, and can also give rise to proteinuria in man, when absorbed over many years, may help to clarify the mechanism of metal poisoning and proteinuria.

Experiments are in progress to locate the source of production of this protein using fluorescent antibody techniques, and also to elucidate possible autoimmune reactions to this protein in the rat, following our observation that the protein disappears from the blood after some 6–8 weeks of thrice-weekly doses of 0·5 mgm. per kgm. of cadmium, administered intraperitoneally.

A full account of this work will be published shortly.

D. J. Lawford

Medical Research Council,
Department for Research in Industrial Medicine,
The London Hospital,
Whitechapel,
London, E.1.

[1] Friberg, L., *Acta med. scand.*, Suppl. 240, 1 (1950).
[2] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[3] Poulik, M. D., *Nature*, **180**, 1477 (1957).
[4] Gedin, H. I., and Porath, J., *Biochim. Biophys. Acta*, **26**, 157 (1957).

Comparison between the Effects of Cysteine and of Anoxia on the Rate of Mitosis in Lens Epithelium

Both anoxia and pretreatment with certain thiols will reduce the sensitivity to radiation of many types of living organisms. The possibility that thiols protect against radiation damage because they lower oxygen tension has been reviewed by Gray[1] and by Pihl and Eldjarn[2]. In spite of all the work done it is not yet clear whether the protective thiols act by inducing tissue anoxia or by some other means.

Pirie and Lajtha[3] found that injection of cysteine into a rabbit inhibited cell division in the lens epithelium, the mitotic inhibition beginning about 18 hr. after injection and lasting four days. von Sallmann[4] had previously shown that cysteine given before irradiation will prevent radiation cataract in the rabbit lens. Since measurement of mitosis in lens epithelium is a simple quantitative method of assessing an effect of cysteine *in vivo*—whether related to protection against radiation or not—we thought it

worth while to compare the mitotic inhibition caused by cysteine with that caused by lack of oxygen.

Rats, aged 7–11 weeks old, were used, and comparisons were made between normal and treated litter mates. Cysteine treatment consisted in a single intravenous injection of 10 per cent cysteine, at a dosage of 100 mgm. cysteine/100 gm. body-weight. The rats were killed at about 8, 18, 24, 32 and 48 hr. after injection. Anoxia was achieved by keeping the rats in an atmosphere of about 5 per cent oxygen, 1 per cent carbon dioxide and 94 per cent nitrogen for up to 18 hr. Rats were killed after 7, 9 and 18 hr. anoxia, and after 18 hr. anoxia followed by 2, 6 or 30 hr. in the normal air. Total mitotic counts were made in whole flat preparations of lens epithelia[5].

The duration of anoxia is definite, but the length of time during which cysteine, plus cystine and other breakdown products, were present in the blood and aqueous humour is not known, nor is the necessary effective concentration. von Sallmann, Dische, Ehrlich and Munoz[6], however, have shown that, in the rabbit given a somewhat smaller intravenous dose of cysteine (70 mgm./100 gm.), the cysteine plus cystine reached maximum levels in the blood and aqueous humour within 1–2 hr., and in 6 hr. had fallen to well below half these values. It is reasonable to assume, therefore, that the length of time during which cysteine and its products were present at effective levels was certainly no longer than the 18-hr. period of anoxia.

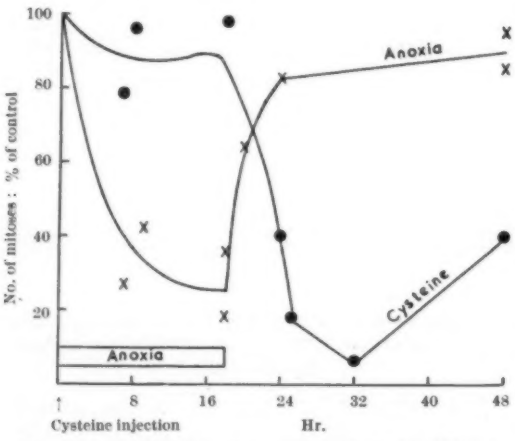


Fig. 1. Comparison between the effects of cysteine (●) and of anoxia (×) on the rate of mitosis in lens epithelium

The effect of cysteine, and of anoxia, on the number of mitoses in the lens epithelium is shown in Fig. 1. There were no damaged cells in any of the preparations. The figure shows that, after 7, 9 and 18 hr. of anoxia, the rate of mitosis had dropped to well below 50 per cent of normal; when the rats were returned to air there was a rapid return to the normal level, recovery being probably complete within 6 hr. With cysteine, on the other hand, there was no significant decrease in mitosis until 24 hr. after the injection, and the rate was still depressed at 48 hr.

Thus in this particular system, and under these particular conditions, the effect of cysteine can be clearly distinguished from that of anoxia.

We thank the National Council to Combat Blindness, New York, for a "Fight for Sight" grant.

Antoinette Pirie
Ruth van Heyningen
Nuffield Laboratory of Ophthalmology,
University of Oxford.

[1] Gray, L. H., "Ann. Rev. Nuclear Sci.", **6**, 353 (1956).
[2] Pihl, A., and Eldjarn, L., *Pharmacol. Rev.*, **10**, 437 (1958).
[3] Pirie, A., and Lajtha, L. G., *Nature*, **184**, 1125 (1959).
[4] von Sallmann, L., *Amer. Med. Assoc. Arch. Ophthal.*, **47**, 305 (1952).
[5] Howard, A., *Stain Technol.*, **27**, 313 (1952).
[6] von Sallmann, L., Dische, Z., Ehrlich, G., and Munoz, C. M., *Amer. J. Ophthal.*, **34**, May, Pt. 2, 95 (1951).

Serotonin, a Melanocyte-stimulating Component in the Dorsal Skin Secretion of *Xenopus laevis*

It is well known that a variety of substances are able to produce a dispersion or concentration of the pigment granules in the melanocytes of Amphibia *in vivo*. However, the number of compounds which produce a dispersion or concentration of these granules in melanocytes of isolated pieces of skin is much smaller. Any secondary reactions may be excluded by this latter *in vitro* method of testing. At the present stage of investigation, *in vitro* studies may be of value, though it should be realized that what happens in Nature may not be comparable.

In a series of investigations, using phenylalkylamines, Burgers[1] found a correlation between melanocyte activity *in vitro* and chemical structure. Furthermore, he described[2] a dispersion of the pigment granules of melanocytes in isolated webs of *Xenopus laevis* after administration of an extract of the dorsal skin secretion of the same species. The purpose of the present investigation was to determine the chemical nature of the active substance in this secretion.

After extraction of dried, defatted skin secretion with methanol, the presence of several indole derivatives could be demonstrated chromatographically, and one of these proved to be active in the *in vitro* test. Catecholamines could not be detected in the extracts. The ultra-violet absorption spectrum of the active indole compound showed a maximum at 275 mμ with a shoulder at 295 mμ, suggesting the presence of a hydroxy-group at position 5 (ref. 3).

The absorption maxima showed an acid-base shift, indicating a free hydroxy-group. This was confirmed by paper electrophoresis at *p*H 13 during which the indole component moved towards the anode. Adsorption of the active compound on 'Amberlite *HCG*-50 (H^+)', migration to the cathode during paper electrophoresis at *p*H 7, and the fluorescence reaction of Shepherd *et al.*[4], suggested the presence of 5-hydroxytryptamine (serotonin) or its derivatives. Identity of the R_F-values of the active indole compound with

Table 1. R_F-VALUES OF THE ACTIVE INDOLE COMPOUND COMPARED WITH SYNTHETIC SEROTONIN

Developing liquid	Active indole compound	Synthetic serotonin
20 per cent aqueous potassium chloride	0·34	0·34
n-Butanol/hydrochloric acid (98 : 2), after impregnating in 0·5 *M* potassium chloride	0·41	0·43
n-Butanol/acetic acid/water (4 : 1 : 5)	0·49	0·49
iso-Butanol-1, saturated with water	0·19	0·22
Phenol/0·1 *N* hydrochloric acid (85 : 15)	0·49	0·48

synthetic serotonin in several chromatographic systems led us to the conclusion that the active compound is identical with serotonin.

Using the *in vitro* test, synthetic serotonin proved to be active. Activity of serotonin with respect to the melanocytes *in vivo* has been described by Davey[5], whereas Wright and Lerner[6] announced a concentrating effect of serotonin *in vitro* in *Rana pipiens*. The presence of serotonin in the venom of *Bufo marinus* has been ascertained by Udenfriend *et al.*[7], who stressed its role as a precursor of the well-known methylated derivatives bufotenine, bufotenidine, etc.

Whether there exists a correlation between the melanocyte activity *in vitro* and the chemical constitution of tryptamine derivatives is being investigated.

I am indebted to Prof. G. J. van Oordt for his interest and encouragement and to Miss A. W. van Wijngaarden, Mr. J. W. Huismans and Mr. A. D. F. Addink for their skilful assistance.

F. C. G. van de Veerdonk

Zoological Laboratory,
Department of Endocrinology,
Janskerkhof 3,
Utrecht.

[1] Burgers, A. C. J., *Acta Physiol. Pharmacol. Neerl.*, **5**, 263 (1957).
[2] Burgers, A. C. J., and van Oordt, G. J., *Acta Endocrinol.*, **23**, 265 (1956).
[3] Stoll, A., Troxler, F., Peyer, J., and Hofmann, A., *Helv. Chim. Acta*, **38**, 1452 (1955).
[4] Shepherd, D. M., West, G. B., and Erspamer, V., *Nature*, **172**, 357 (1953).
[5] Davey, K. G., *Nature*, **183**, 1271 (1959).
[6] Wright, M. R., and Lerner, A. B., *Nature*, **185**, 169 (1960).
[7] Udenfriend, S., Clark, C. T., and Titus, E., *Experientia*, **8**, 379 (1952).

Sodium and Potassium in Mitochondrial Membrane Fractions

In a preliminary attempt to explain the observations that muscle[1] and liver[2] mitochondria are able to transport actively potassium but not sodium, the concentrations of these ions in mitochondrial membrane fractions were studied and compared with those in intact mitochondria. The results indicated that the specific activity (μgm. sodium or potassium/mgm. dry weight) of sodium was several times greater in mitochondrial membrane fractions than in intact particles, whereas the reverse was found with potassium.

Heart mitochondria were isolated from 6–9 lb. male rabbits as described previously[3], and rabbit liver and kidney mitochondria were prepared as described by Hogeboom[4]. Mitochondria were washed three times with the same fluid used in homogenizing the tissues and then suspended with 0·25 *M* sucrose. To 9 ml. of the mitochondrial suspension, 1 ml. of a 3 per cent solution of deoxycholic acid was added and the mixture rapidly stirred with a glass rod until it became clear. The deoxycholic acid solution was freshly prepared for each experiment by adding 15 ml. water to 750 mgm. deoxycholic acid (Mann Research Laboratories), and then solubilizing the acid by stirring in 1·4 gm. *tris* [*tris*(hydroxymethyl)aminomethane] buffer. The *p*H of the solution was adjusted to 7·6 by slowly adding 5 *N* hydrochloric acid and then diluting to 25 ml. with water. To mitochondria which were not disrupted, 1 ml. of either 0·25 *M* sucrose or 0·46 *M* *tris* buffer (*p*H 7·6) was added. The rest of the procedure was similar to that described[5] for isolating a membrane fraction from 0·44 *M* sucrose suspensions of rat liver mitochondria. The pellet obtained by centrifugation of the 25,000*g* supernatant for 1 hr. at 105,000*g* was called the membrane fraction. Dry-weight determinations and analyses of sodium and potassium were carried out as described previously[1].

Table 1. Sodium and Potassium Contents of Intact Mitochondria and Mitochondrial Membrane Fractions

Experiment	Sodium		Potassium	
	Intact mito.	Membrane	Intact mito.	Membrane
	(μgm.Na/mgm. dry weight)		(μgm.K/mgm. dry weight)	
Liver				
(1) 0·44 *M* sucrose susp.	0·14	1·66	1·05	—*
(2*a*) 0·3% DOC	0·26	0·88	5·15	0·97
(2*b*) 0·5% DOC	0·26	1·66	5·15	1·06
(3*a*) 0·3% DOC	0·34	0·67	4·43	0·45
(3*b*) 0·6% DOC	0·34	1·46	4·43	1·27
(4*a*) Fresh	0·20	1·66	4·99	0·75
(4*b*) Aged 4 days	0·21	1·42	0·56	0·74
(5) Water suspension, sonicated	0·97	1·78†	0·73	0·83†
(6*a*) 0·3% DOC	0·21	1·56	4·52	0·86
(6*b*) Sucrose suspension, sonicated	0·21	4·02	4·52	3·79
Kidney				
(7*a*) 0·3% DOC	0·27	0·66	2·22	0·44
(7*b*) 0·5% DOC	0·27	1·01	2·22	0·61
(8*a*) Membrane unwashed	0·18	0·49	1·48	0·30
(8*b*) Membrane washed 1×	0·18	0·58	1·48	0·22
(9*a*) Control	—	—	1·72	1·19
(9*b*) ATP + α-ketoglutarate	—	—	2·97	1·10
Heart				
(10*a*) 0·3% DOC	0·22	0·73	1·88	0·39
(10*b*) 0·5% DOC	0·22	1·14	1·88	0·61
(11*a*) Membrane unwashed	0·17	0·68	2·62	0·61
(11*b*) Membrane washed 2×	0·17	0·72	2·62	0·54
(12*a*) Fresh	0·31	1·37	0·86	0·57
(12*b*) Aged 4 days	0·17	1·51	0·32	0·78

Unless otherwise indicated, mitochondria were suspended with 0·25 *M* sucrose and treated with deoxycholic acid (DOC) to give a final concentration of 0·3 per cent. All figures are usually the mean of 3 individual determinations.
* Too little potassium for determination.
† Mean of six determinations.

Table 1 presents a summary of the results obtained from experiments with liver, kidney, and heart mitochondria. Although results were also obtained for the sodium and potassium contents of the fraction isolated by centrifugation of the mitochondria treated with deoxycholic acid for 20 min. at 25,000*g*, they were not included because this fraction was not homogeneous and probably contained mostly mitochondria which were partially disrupted[5]. The specific activity of the sodium and potassium in this fraction ranged from 0·19 to 4·87 and from 0·15 to 1·98, respectively.

The results in Table 1 demonstrate that the specific activity of sodium in the membrane fractions from all three kinds of mitochondria treated with deoxycholic acid was 2–12 times greater than that in the intact mitochondria. The specific activity of potassium, however, was 1·5–10 times greater in the fresh intact particles than in the membrane fractions. Washing the membrane fraction once or twice with isotonic sucrose (Exps. 8 and 11) appeared to remove little, if any, potassium or sodium. In four experiments, a comparison was made between the ion contents of membrane fractions obtained from 0·3 per cent and 0·5 per cent (or 0·6 per cent) particles treated with deoxycholic acid since the higher concentration has been found to result in closely apposed membranes in contrast to the vesicular elements observed in the electron microscope after treatment with 0·3 per cent deoxycholic acid[5]. The results showed (Exps. 2, 3, 7, and 10) that the specific activity was significantly increased, generally more so for sodium than for potassium, with 0·5 per cent deoxycholic acid. Part of this increase in specific activity was probably a

result of the decreased dry weight of the membrane fraction after treatment with 0·5 per cent deoxycholic acid, the average dry weights decreasing from 12·6 to 7·8 mgm. for liver, from 17·4 to 10·7 mgm. for kidney, and from 13·6 to 11·6 mgm. for heart.

In order to determine whether the increase in the potassium gradient, which occurs when mitochondria are incubated in a medium to which an adenine nucleotide and oxidizable substrate are added, is due to an increased amount of the ion in the membrane fraction or in the rest of the mitochondrial water, kidney mitochondria were incubated in a medium (0·25 *M* sucrose, 0·005 *M* magnesium sulphate, 0·005 *M* potassium phosphate buffer, *p*H 7·4, 0·005 *M* sodium phosphate buffer, *p*H 7·4) with or without the addition of 0·001 *M* adenosine triphosphate and 0·02 *M* α-ketoglutarate. After 20 min. incubation in air at 37°, deoxycholic acid was added to half the mitochondrial suspensions to give a final concentration of 0·3 per cent and the membrane fraction was isolated as described previously. Untreated suspensions were centrifuged[1] to separate the intact mitochondria from the suspending medium. The results showed that the potassium gradient:

$$\frac{\text{m.equiv. potassium/litre mitochondrial water}}{\text{m.equiv. potassium/litre suspending medium}}$$

increased from 1·56 to 2·83 when adenosine triphosphate and α-ketoglutarate were added to the medium, the specific activity of potassium increasing from 1·72 to 2·97 (Exp. 9). Since the specific activity of the membrane fraction of the two groups was practically the same, this experiment demonstrates that the increased potassium which is accumulated does not concentrate in the membrane. The rather small difference in specific activity between the intact mitochondria and the membrane fraction in this experiment may be a consequence of suspending the particles in a relatively potassium-rich medium for incubation.

The deoxycholic acid used in these experiments contained considerably less than 1 μgm. potassium/1 ml. solution, but it did contain 5 μgm. sodium/1 ml. solution. Since the total sodium in the membrane fraction was usually 5–15 μgm., a significant error would be introduced into the calculation of specific activity if some or all of the sodium from the acid became attached to the membrane fraction. In attempting to demonstrate that the differences in sodium concentration between the intact mitochondria and the membrane fraction were not an artefact, liver mitochondria suspended either in water or 0·25 *M* sucrose were subjected to ultrasonic vibrations for 1–5 min. in a 10 kc./s. Raytheon sonic oscillator at 250 watts and 1·25 amp. The ultrasonically treated particles were centrifuged for 20 min. at 25,000*g* and the resulting supernatant was centrifuged for 1 hr. at 105,000*g* to isolate a membrane fraction[6,7]. Although mitochondrial membranes isolated from sonically disrupted mitochondria apparently are not pure, in that matrix adheres to the membrane vesicles[5], the inhomogeneity of the membrane fraction probably would not affect markedly the specific activity of the sodium, especially since it is so low in the intact mitochondria. The results, although quantitatively different (Exps. 5 and 6), tended to confirm those obtained with deoxycholic acid. The very low potassium specific activity of the intact water-suspended mitochondria (Exp. 5) was undoubtedly due to leakage of the ion from the swollen particles since the supernatant after isolation of the mitochondria contained very much larger amounts of the ion than were found in the supernatant of 0·25 *M* sucrose-suspended mitochondria.

The data obtained from this work may provide an explanation for the observation that when muscle mitochondria are aged, the potassium content decreases approximately 50 per cent, whereas the sodium content remains virtually unchanged[1]. The decrease in potassium might be explained if one assumes that the small quantity of membrane potassium is firmly bound (see Exps. 8 and 11), while the rest of the mitochondrial potassium is either not bound or is bound to compounds which leak out of the particles during ageing. Since most of the mitochondrial sodium appears to be concentrated in the membrane fraction, ageing would not affect it, if one again assumed that it was tightly bound. In this connexion, the sodium and potassium in the membrane fraction of mitochondria aged for four days by suspending them in 0·25 *M* sucrose at 0° do not appear to change significantly (Exps. 4 and 12).

Whether the differences in sodium and potassium concentration in the mitochondrial membrane fraction have any connexion with the inability of muscle[1] and liver[2] mitochondria to transport sodium against a concentration gradient cannot be assessed at the present time. Further work on this problem is in progress.

This investigation was supported by grants from the National Institute of Arthritis and Metabolic Diseases and the American Heart Association. I am grateful for the very skilful technical assistance of Mrs. Patsy L. S. Smith and Mr. Arthur Nathanson.

Frank Ulrich*

Department of Physiology,
Yale University School of Medicine,
New Haven 11, Conn.

* Established Investigator of the American Heart Association.

1 Ulrich, F., *Amer. J. Physiol.*, **197**, 997 (1959).
2 Spector, W. G., *Proc. Roy. Soc.*, B, **141**, 268 (1953).
3 Ulrich, F., *Amer. J. Physiol.*, **198**, 847 (1960).
4 Hogeboom, G. H. in "Methods in Enzymology" ed. by S. P. Colowick and N. O. Kaplan, **1**, 16 (Academic Press, N.Y., 1955).
5 Watson, M., and Siekevitz, P., *J. Biophys. Biochem. Cytol.*, **2**, 639 (1956).
6 McMurray, W. C., Maley, G. F., and Lardy, H. A., *J. Biol. Chem.*, **230**, 219 (1958).
7 Kielley, W. W., and Bronk, J. R., *J. Biol. Chem.*, **230**, 521 (1958).

Antigonadotropic Function of the Pineal Gland and the Oxytocin of the Neurosecretory Hypothalamic System

The existence of an active substance in the pineal gland capable of inhibiting gonadotropic hormones is supported by most authors who have studied this problem. On the other hand, nothing has been revealed concerning the mechanism which causes this inhibition. The existence of a direct interaction between the pineal-inhibiting factor and gonadotropic hormones does not seem likely. In connexion with investigations carried out on anti-androgenic fractions of bovine pineal gland, one of us claimed that the antigonadotropic function of the pineal gland was exerted at the level of the subcortical centres inhibited by it[1].

On the other hand, the work of Shibusawa *et al.*[2] and Martini *et al.*[3] seems to indicate that oxytocin represents the releasing factor for gonadotropic hormones.

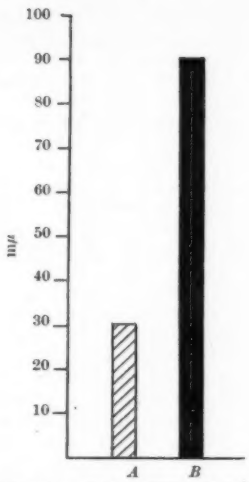


Fig. 1. Oxytocic activity of dog paraventricular nucleus (mean values of 6 animals). *A*, normal activity (30 ± 9 mμ); *B*, 5 hr. after administration of pineal extract (90 ± 12 mμ). The means and their standard errors are given as mμ, in terms of U.S. Pharmacopœia reference standard

If this hypothesis is correct, the antigonadotropic function of the pineal gland ought to be exerted through the blocking of the oxytocin as a releasing factor in the nuclei of the neurosecretory hypothalamic system.

Our experiment was performed on 12 adult male dogs weighing about 15 kgm. The animals were divided into 2 groups: 6 animals served as controls and another 6 were given intravenous injections of 10 ml. protein extract of bovine pineal gland containing a nitrogen equivalent of 0·03 mgm./ml. The animals were killed 5 hr. after the administration of the pineal extracts, with as little psychic disturbance as possible, by the rapid intravenous injection of magnesium as a 25 per cent w/v aqueous solution of the sulphate. The whole brain and pituitary body were removed in about 10 min. and placed in 20 vol. of anhydrous acetone. The supra-optic nuclei, the paraventricular nuclei, the tuber cinereum and the neurohypophysis were isolated. Extraction was performed according to the method of Van Dyke *et al.*[4]. Holton's method[5] was used for the assay of oxytocin. All determinations were performed in comparison with U.S. Pharmacopœia posterior pituitary reference standard.

The normal amounts of oxytocin that we found in the paraventricular nucleus is in strong agreement with those of Van Dyke *et al.*[4].

Five hr. after the intravenous administration of 10 ml. protein extract of bovine pineal gland, the oxytocic activity of the paraventricular nucleus increased from 30 ± 9 to 90 ± 12 mμ (Fig. 1). We consider this increased concentration of oxytocin to be due to blocking of this polypeptide under the action of the pineal extract.

In a recent paper[6], a very low oxytocic activity was described in the nuclei of the neurosecretory hypothalamic system during the releasing of gonadotropic hormones. In the present work, concomitantly with the blocking of gonadotropic hormones, there is a significant increase of the oxytocic activity in the nuclei of the neurosecretory hypothalamic system. This parallelism between the releasing or blocking of gonadotropic hormones and releasing or blocking respectively of oxytocin in the neurosecretory hypothalamic system supports the hypothesis of Shibusawa and Martini according to which oxytocin or an active substance associated with it represents the releasing factor for the gonadotropic hormones. On the other hand, this experiment shows that the antigonadotropic function of the pineal gland is achieved through blocking of the oxytocin or of an active substance associated with it in the neurosecretory hypothalamic system, and not through the direct interference with gonadotropic hormones. The antigonadotropic function of the pineal gland is in fact an antigonadotropic hormone function. This hypothesis is strongly supported by Milin's histophysiological investigations[7]. He found a lowered activity of the nuclei of the neurosecretory hypothalamic system in the rabbit during the blocking of the gonadotropic hormones by darkness and an increased activity of these nuclei during the releasing of gonadotropic hormones caused by light. In the first case, the pineal gland is hyperactive, in the second hypoactive. Investigations which are under way concerning the action of pineal extracts on vasopressin in the neurosecretory hypothalamic system make us assume that the anticorticotropic function of the pineal gland is exerted through the intermediary of vasopressin as a releasing factor of a substance associated with it, and not through the direct interference with adrenocorticotropic hormone.

Thus, it seems that the antigonadotropic mechanism consisting in the blocking of releasing factors represents a general pattern according to which the pineal gland exerts its antitropic functions. Investigations now being carried out will, we hope, furnish new data on the relationship between tropic hormones and the releasing factors in the neurosecretory hypothalamic system.

S.-M. Milcou
S. Pavel

Institute of Endocrinology,
Bucharest.

[1] Milcou, S.-M., "Epifiza glandă endocrină", Cong. Nat. Sci. Med., Bucuresti (May, 1957).
[2] Shibusawa, K., Saito, S., Fukuda, M., Kawai, T., Yamado, K., and Tomizawa, K., *Endocrinol.* (Japan), **2**, 3 (1955).
[3] Martini, L., Mira, L., Pecile, A., and Saito, S., *Acta Endocrinol.*, Supp. 38 (1958).
[4] Van Dyke, H. B., Karlis, Adamson, jun., and Stanford, L. Engel, in "Recent Progress in Hormone Research", 1 (Academic Press, New York, 1955).
[5] Holton, P., *Brit. J. Pharm. Chem.*, **3**, 328 (1948).
[6] Milcou, S.-M., Pavel, S., and Pterescu, S., *Rumanian Med. Rev.* (in the press).
[7] Milin, R., Cong. Nat. Sci. Med. Bucuresti (May, 1957).

Autonomic Nerve–Smooth Muscle Transmission

Despite extensive work on the morphology and pharmacology of autonomic effector systems, very little is known about the mechanism of transmission of excitation from nerve to smooth muscle. In the present work our approach has been to apply modern electrophysiological methods to a suitable autonomic nerve–smooth muscle preparation in a manner similar to that described for the skeletal neuro-muscular junction[1].

Smooth muscle cells from the longitudinal layer of the vas deferens of the guinea pig were impaled with capillary microelectrodes and membrane potential

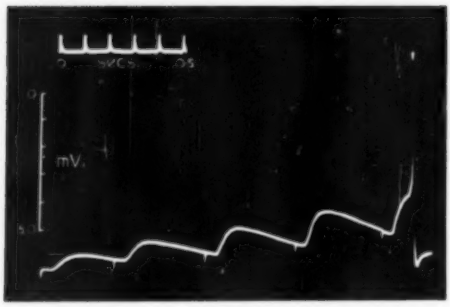


Fig. 1. Membrane potential recorded from a single smooth muscle fibre of the vas deferens of the guinea pig during repetitive stimulation of the hypogastric nerve (stimuli 1 msec. duration). Successive 'junction potentials' increase in amplitude and rate of rise. Summation occurs until at a critical depolarization a spike is initiated

changes recorded during repetitive stimulation of the hypogastric nerve. The vas deferens was never spontaneously active. In the experiment illustrated in Fig. 1, the resting potential was 65 mV. Five successive stimuli resulted in depolarizations of progressively greater amplitude until, at a critical membrane potential (37 mV.), a spike was initiated and a vigorous contraction occurred. The membrane potential at which the spike arose was remarkably constant for the majority of cells (35–38 mV.). The number of stimuli required to reach this threshold, however, was variable.

The mean resting potential of the vas deferens was 57 mV. (SE_M = 6 mV.). The spike was similar in configuration to those recorded from the taenia coli of the guinea pig[2] and the uterus of the rat[3]. Spike amplitudes of up to 84 mV. were recorded (mean 68 mV., SE_M = 8 mV.); overshoots were up to 20 mV. The positive phase of the spike (after-hyperpolarization) generally brought the membrane potential back to, or beyond, its resting-level.

We have decided to call the successive depolarizations in response to nerve stimulation 'junction potentials'. We felt that the term 'end-plate potential' was best reserved for tissues where a definite end plate region could be distinguished; the term 'post synaptic potential' did not seem appropriate since, at present, there is no histological evidence for any structure resembling a neuronal synapse in smooth muscle.

Both the amplitude and the rate of depolarization of the junction potentials increased with successive stimuli, this facilitation occurring even when the interval between stimuli exceeded 500 msec. The rate of depolarization of the junction potential was slow compared with other post-synaptic potentials. The rate of decay of the junction potential varied greatly from preparation to preparation. We think it is unlikely that this slow decay is dependent on the time-constant of the membrane, but that it may represent slow destruction of the transmitter. Brown and Gillespie[4] suggested that at least 100 msec. were required for destruction of the noradrenaline released from cat spleen by splenic nerve stimulation.

Junction potentials could be recorded in cells along the entire surface of the vas deferens. Their amplitude and latency varied somewhat from cell to cell, but there was no indication of a defined end-plate region. The functional distribution of nerve endings therefore appears to resemble that of crustacean muscle[5]. The amplitude of the junction potentials was dependent on the number of nerve fibres stimulated. If the hypogastric nerve was stimulated submaximally, the amplitude of junction potentials all over the muscle was reduced.

In the absence of nerve stimulation small 'miniature junction potentials' were recorded which presumably indicated the spontaneous release of transmitter. Their frequency was lower than that observed at the skeletal neuromuscular junction[6]. Their amplitude was larger (up to 18 mV.); but this may be accounted for in terms of the small diameter of the muscle fibres[7].

The response of the vas deferens to hypogastric nerve stimulation was unaffected by hexamethonium (5×10^{-5}) and atropine (10^{-6}), but was blocked by the anti-adrenergic drug darenthin (5×10^{-5}). Thus, stimulation appears to have been post-ganglionic and the transmitter is most likely to be noradrenaline.

These results will be published in detail elsewhere.

We wish to thank Dr. Michael Rand for introducing us to this preparation and for assisting us with the pharmacology.

G. Burnstock
Mollie E. Holman

Departments of Zoology and Physiology,
University of Melbourne,
Victoria.

[1] del Castillo, J., and Katz, B., "Progress in Biophysics", **6**, 122 (Pergamon Press, London and New York, 1956).
[2] Holman, Mollie E., *J. Physiol.*, **141**, 464 (1958).
[3] Marshall, Jean M., *Amer. J. Physiol.*, **197**, 935 (1959).
[4] Brown, G. L., and Gillespie, J. S., *J. Physiol.*, **138**, 81 (1957).
[5] Fatt, P., and Katz, B., *J. Exp. Biol.*, **30**, 433 (1953).
[6] Boyd, I. A., and Martin, A. R., *J. Physiol.*, **132**, 61 (1956).
[7] Katz, B., and Thesleff, S., *J. Physiol.*, **137**, 267 (1957).

PSYCHIATRY

Paradoxical Blocking and Arousal in the Drowsy State

There have been many attempts to investigate the hypnotic state making use of the electroencephalograph. Though clinically the subject might have the appearance of sleep, the electroencephalograph was not found to be different from that found in the wide-awake hypnotized state. Darrow *et al.*[1] believed that the electroencephalograph in hypnosis had characteristics that distinguished it from both true sleep and full consciousness. Our experience does not confirm either of these views, but we have not found the electroencephalograph of a hypnotized person to have any distinctive characteristics. Rather the electroencephalograph of the hypnotized person can be characteristic of the waking or drowsing electroencephalogram without hypnosis, depending upon the situation and the suggestions given. This finding is therefore in accord with those of older writers on the subject, including Paul Schilder[2].

In the case we are describing, the patient had been hypnotized several times. Suggestions were given to "Allow yourself to go deeper and deeper asleep . . ." with explanations to the effect that the word 'sleep' was here being used in a special sense, because the subject was told that he could still hear and comprehend the experimenter. On other occasions he was urged to go to sleep and become "oblivious to everything and be completely relaxed". On one such occasion when the record gave evidence of drowsiness

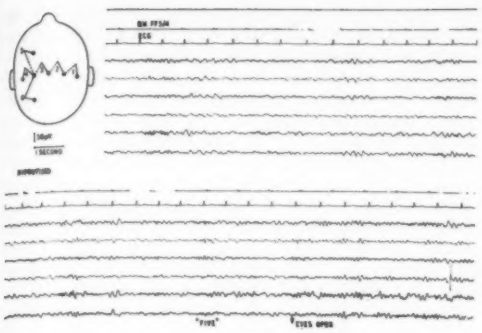


Fig. 1

(in this instance for 40 sec.) it was suggested that after the experimenter had counted from 1 to 5, he would be able to open his eyes. No change in the electroencephalograph occurred during counting or while the instruction was being given. Three seconds after the count of 5 the patient opened his eyes and there was at this time paradoxical blocking of the alpha rhythm (that is, the alpha appeared on eye opening). Despite the fact that the instruction was obeyed, arousal from the drowsy state, as illustrated by the electroencephalogram, did not occur until the patient actually opened his eyes. Therefore opening the eyes was the first external change observed, and the electrical change coincided with this. Since the instruction was obeyed, one would have expected the record to change while the instruction was being given. On other occasions different tasks had been set, to be obeyed on counts of 3 or 5; it is therefore unlikely that on this occasion the correct response was a chance occurrence. There was one other occasion when the patient was instructed to rub his face with his hand after a count of 5. During the instruction the record was that of drowsiness, but when he started to rub his face some 6 sec. after the count of '5', the alpha rhythm appeared. The alpha rhythm here is therefore an index of arousal. The arousal occurred when the instruction was obeyed and not when it was given. This is of interest in view of the finding by Oswald *et al.*[3] that in light sleep *K*-complexes occurred more often to certain names, especially the subject's name, rather than to meaningless sounds. They concluded that the censorship controlling arousal was in the cortex. On the other hand, our example seems to pose again the question asked by Adrian[4]: "If patterns arrive at the cortex, who reads the patterns?" Adrian[5] also said, "The meaning of the stimulus is there before the mind comes to understand it". Our example perhaps supports this statement, the instruction having been received and then apperceived, and the stimulus for arousal comes into being as a result of the significance of the instruction for the sleeper. We would agree more with Adrian[5] that the diencephalon is mostly concerned. "The physical intensity of the stimulus is not as important as the emotional associations"[5]. This statement is supported by our observation. The instruction is received and the mechanism of response put in train (? understood). Electroencephalograph arousal, when it occurs, is not therefore to the auditory stimulus of the instruction given nor to the internal mechanism set in train to provide the ultimate response, but takes place when this response occurs. Arousal is then a response to the emotion evoked by the performance of the act itself and the meaning of it to the sleeper.

L. Goldie
J. M. Green

Department of Clinical Neurophysiology,
Institute of Psychiatry,
Maudsley Hospital,
London, S.E.5.

[1] Darrow, C. W., Henry, C. E., Brenman, M., and Gill, M., *EEG. Clin. Neurophysiol.*, **2**, 231 (1950).
[2] Schilder, P., "The Nature of Hypnosis", trans. by Gorvin, G. (International Universities Press, New York, 1956).
[3] Oswald, I., Taylor, A., and Treisman, M., *EEG. Clin. Neurophysiol.*, **11**, 603 (1959).
[4] Adrian, E. D., in "Brain Mechanisms and Consciousness", edit. by Delafresnaye, J. F., 237 (Blackwell Scientific Publications, 1954).
[5] Adrian, E. D., *Irish. J. Med. Sci.*, **138**, 237 (1937).

RADIOBIOLOGY

Dye-sensitized Photo-reactivation of X-ray Damage in Diploid Yeast

In an investigation of the binding of amino-acridine dyes to deoxyribonucleic acid in living cells we have shown that the nucleic acid fluorochrome, acridine orange (3,6,-*bis*-dimethylaminoacridine), sensitizes both haploid and diploid yeast (*Saccharomyces cerevisiae*) to visible light[1]. The significance of the survival curves obtained for this acridine orange-sensitized photo-inactivation was further examined by experiments involving additivity of X-rays, ultra-violet and visible light. In the course of these additivity studies[2] a new effect appeared: dye-sensitized visible-light reactivation of X-ray damage in diploid cells. This effect is of special interest because, although ultra-violet damage having a nucleic-acid action spectrum is partially reversible by visible light, previous attempts at reactivation of X-ray damage with visible light have not been successful[3].

The techniques for handling and irradiating the yeast have been described elsewhere[4]. In the present experiments cells which had grown for seven days at room temperature on potato dextrose agar were suspended in *p*H 7 phosphate buffer to make a final concentration of 10^6 cells/ml. This suspension was exposed first to a dose of X-rays (dose-rate 330 r./min.) sufficient to inactivate a predetermined fraction of the population. Then purified acridine orange (1 : 2,000) was added to make a final dye concentration of 1 : 200,000 or $1{\cdot}8 \times 10^{-5}\,M$; after 20 min. in the dye the cells were irradiated with visible light (10^5 ergs/cm.^{2}/sec.). The light source was a General Electric *R*-52 750-watt flood lamp. The output of this lamp was filtered through a copper sulphate solution and a Corning filter No. 3389 to remove infra-red and near ultra-violet components which cause inactivation in the absence of the dye.

The results of a typical experiment are shown in Fig. 1. Here, the suspension was irradiated with X-rays until 85 per cent of the original population was inactivated (that is, to the 15 per cent survival-level). (Similar results are obtained for both lower and higher levels of survival.) In all cases, subsequent irradiation with low doses of visible light (in the presence of dye) increases this surviving fraction, thus indicating a repair or by-pass of part of the damage produced by the X-rays. With increasing dose, simultaneous killing by the visible light first lessens and finally overrides the observed reactiva-

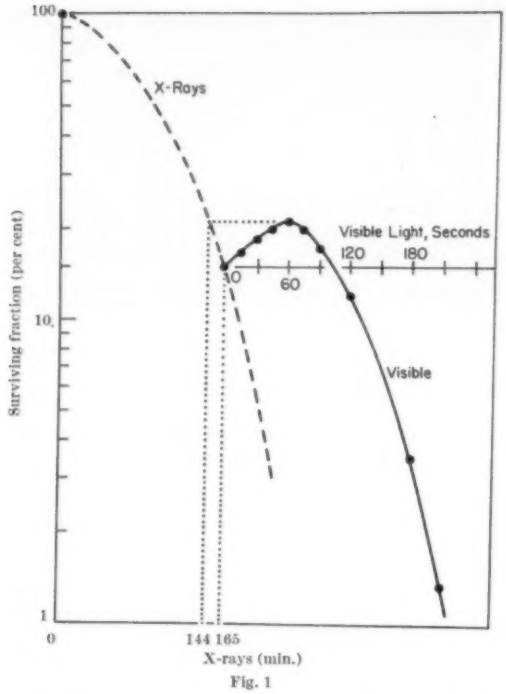


Fig. 1

tion. At the apparent maximum (60 sec.), the dose reduction factor for X-ray damage is 165/144 = 1·14. The actual maximum degree of reactivation is probably much higher and could ordinarily be determined graphically by subtracting the normal visible light inactivation curve, were it not for the quantitatively unknown degree of additivity of X-rays and visible light. (The visible light inactivation curve which would occur in the absence of reactivation is steeper than the ordinary curve for a non-pre-irradiated population because of the existence of common sites for X-ray and visible light damage.)

This reactivation of X-ray damage is not found in haploid yeast. Since the dye-sensitized visible light survival curve for haploid cells surviving X-irradiation is identical with that for non-pre-irradiated cells, there is not only no increase in survival, but also no interaction of the two irradiations at all. This lack of haploid reactivation suggests some difference between the inactivation process for the two ploidies that is not necessarily associated with simple genetic duplication. Clearly, either the reactivable site in the diploid accounts for, at most, a very small part of the X-ray damage in the haploid (which implies some independence in the X-ray sites of the haploid and diploid), or the site is present in both haploid and diploid; but, because of some difficulty in the dye-binding or sensitization process, it is not reactivable in the former. It is difficult to separate these possibilities, since from additivity and survival experiments we have been able to conclude only that for X-rays and visible light (with dye) some of the haploid sites are also diploid sites and some of the X-ray sites are also visible sites; we have not yet been able to determine the degree of overlap or to equate one part with another. Reactivation by visible light involving a dye which binds deoxyribonucleic acid nevertheless strengthens the view that part of the X-ray damage to a cell is damage to deoxyribonucleic acid molecules.

It has been reported that X-ray damage in yeast can also be repaired, in part, by ultra-violet light[5]. In this case, also, there is a decreased effect in the haploid, although in a genetically related non-respiring haploid the effect is the same as in the diploid. However, since ultra-violet reactivation of X-ray damage is found only with actively budding cells rather than the resting cells used in the present work, a comparison is probably not valid, especially since there are striking differences in the X-ray curves for resting and budding populations[6].

This investigation was aided by research grant *C*-2739 from the National Cancer Institute of the United States Public Health Service. One of us (D. F.) was supported by a National Science Foundation pre-doctoral fellowship.

DAVID FREIFELDER*
ROBERT B. URETZ

Committee on Biophysics,
University of Chicago,
Chicago 37, Illinois.

* Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Freifelder, D., and Uretz, R. B., *Nature*, **186**, 731 (1960).
Freifelder, D., Ph.D. thesis, University of Chicago (1959).
Jagger, J., *Bact. Rev.*, **22**, 99 (1958).
Uretz, R. B., *Rad. Res.*, **2**, 240 (1955).
Elkind, M. M., and Sutton, H., *Science*, **128**, 1082 (1958).
Beam, C. A., *Rad. Res.*, Supp. **1**, 372 (1959).

Influence of Localized Ultra-violet Irradiation on the Incorporation of Adenine-8-^{14}C and D,L-Methionine-^{35}S in *Acetabularia mediterranea*

WE have shown recently[1,2] that methionine labelled with sulphur-35 is incorporated in a decreasing apico-basal gradient in the unicellular alga *Acetabularia mediterranea*. The existence of this gradient suggested a possible relationship between methionine incorporation, ribonucleic acid metabolism and the production of morphogenetic substances in this alga. We have now examined the effects of localized ultra-violet irradiation, delivered to algal stem tips or rhizoids, on morphogenesis and adenine and methionine incorporation, in order further to evaluate the reality of this relationship and its implications.

The present observations were made on whole algae shortly before cap formation. Irradiations were limited to either the first 3 mm. of stem tip or the rhizoid. The ultra-violet dose applied was equivalent to 8×10^3 ergs/mm.^{2}. This dose, while effective, produces no demonstrable lethality[3]. Incubation with labelled precursors (adenine-8-^{14}C at 0·1 μc. and 0·02 μ*M*/ml., D,L-methionine-^{35}S at 0·1 μc. and 0·002 μ*M*/ml.) occurred over a 48-hr. period immediately following irradiation. Incorporated radioactivity was detected autoradiographically by the methods described by Ficq[4].

While some retardation and partial inhibition of morphogenesis was noted regardless of the point of irradiation, the inhibition of cap formation was considerably more pronounced when the tip was irradiated (about 50 per cent) than when the irradiation was restricted to the rhizoid (about 14 per cent

inhibition). Quantitative analysis of the autoradiographs of unirradiated algae showed an adenine-^{14}C incorporation gradient which was qualitatively similar to that observed with methionine[3,6], but which differed in sharpness; methionine incorporation was ten times more intense in tip cytoplasm than in the cytoplasm of the rhizoid; the adenine incorporation ratio between the two morphological extremities was only 3 : 1.

Irradiation of the rhizoid led to a marked reduction (about 50 per cent) in the incorporation of both adenine and methionine, without modifying the characteristic apico-basal gradients of either; the inhibition produced by rhizoid irradiation was thus a general one. Irradiation of the stem tip, however, resulted in a specific inhibition of adenine and methionine incorporation in the irradiated region; the cytoplasm of the rhizoid was even more strongly labelled after stem tip irradiation than in control algae.

These results suggest that the rhizoid, or more probably the nucleus which is located there, exerts a control, direct or indirect, on the synthesis of ribonucleic acid[5] and that of sulphur-containing proteins[1,2]; these proteins and ribonucleic acid accumulate in the apical extremity of the alga. Ultra-violet irradiation restricted to the apical region inhibits both morphogenesis (that is, cap development) and the accumulation of these substances at the stem tip. These new results provide further support for the notion that ribonucleic acid and sulphur-containing proteins play an important part in the morphogenesis of *Acetabularia mediterranea*[6].

J. Brachet
M. J. Olszewska*

Laboratoire de Morphologie animale,
Université libre de Bruxelles.

* Fellow of the Rockefeller Foundation. Present address: Laboratory of Plant Anatomy and Cytology, University of Łódz, Łódz, Poland.

[1] Olszewska, M. J., and Brachet, J., *Arch. Internat. Biochem.* (in the press).
[2] Olszewska, M. J., and Brachet, J., *Exp. Cell Res.* (in the press).
[3] Errera, M., and Vanderhaeghe, F., *Exp. Cell Res.*, **13**, 1 (1957).
[4] Ficq, A., *Arch. Biol.*, **66**, 506 (1955).
[5] Stich, H., and Plaut, W., *J. Biophys. Biochem. Cytol.*, **4**, 119 (1958).
[6] Brachet, J., *Exp. Cell Res.*, Supp. 6, 78 (1959).

Identification of Transplanted Bone Marrow Cells in Radiation Chimæras

The beneficial effect of bone marrow transfusion in acute radiation syndrome is now well established. This effect seems to depend on the survival of transfused cells and their proliferation in the depleted hæmopoietic tissues of the recipient[1]. In view of this, there arises the problem of identification of donor cells. Various methods were devised to this end, each permitting the recognition of a certain percentage of a particular cell series[1].

The purpose of this work was to find if rabbits, complete heterozygous carriers of the Pelger anomaly, may be used as donors and if transfused cells may be identified by their characteristic morphological appearance. This consists in specific structure of chromatin in all nucleated blood and bone marrow cells, early blast stages and orthochromatic erythroblasts excepted. Moreover, the granulocytes and megakaryocytes are characterized by hyposegmentation of their nuclei[2].

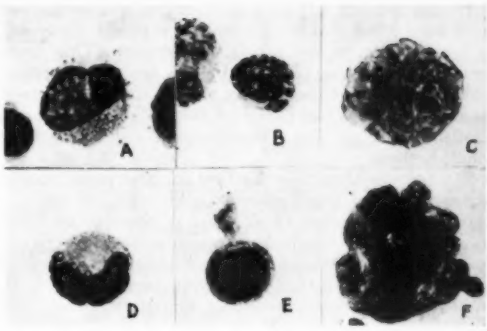


Fig. 1. A comparison between Pelger and normal bone marrow cells. Pelger cells: *A*, Metamyelocyte; *B*, polychromatic erythroblast; *C*, naked megakaryocytic nucleus; *D*, *E*, *F*, normal counterparts. Photomicrograph from a bone marrow smear from a radiation Pelger chimæra

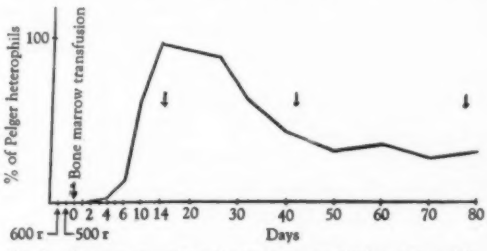


Fig. 2. Curve representing the percentage of Pelger heterophils in the peripheral blood of a Pelger radiation chimæra. 100 per cent = all heterophils in the blood. Arrows indicate bone marrow biopsy

39 rabbits were exposed to total-body X-irradiation (1,100 r.: initial dose of 600 r., followed after 24 hr. by 500 r.). To 5 rabbits an injection of bone marrow cell suspension obtained from Pelger rabbits was administered; 16 animals (males) were injected with bone marrow cell suspension from female donors according to the technique described by Porter[3]. The remaining rabbits served as controls, their mean survival-time being 10 days.

In the first experimental group all donors exhibited Pelger traits in all nucleated blood and bone marrow cells, especially in the granulocytic erythroblastic and megakaryocytic series (Fig. 1), with the above-mentioned exceptions. All recipients were examined beforehand for the presence of Pelger traits, because of the frequent occurrence of partial carriers among rabbits[4]. Following successful bone marrow transplantation, donor cells could be easily identified in peripheral blood (especially heterophils, Fig. 2) of the recipients. We obtained two rabbit-radiation-Pelger-chimæras, which survived longer than 30 days. Fig. 2 illustrates the findings in one of these (peripheral blood). Donor cells could be identified also in the bone marrow, which may be illustrated by the findings of bone marrow biopsy performed the fourteenth day on the same animal, as in Fig. 2.

Table 1

Cell series (per cent)	Granulocytic	Erythroblastic	Megakaryocytic
Pelger (donor) cells	82	46	46
Host's cells	8	34	33
Unidentified cells	10	20	21

In the second experimental group the transfused cells were identified by the presence of sex chromatin in heterophils, as described by Porter[3]. This group served as controls for the Pelger group, showing a similar survival-time. The time-course of increase in donor cells in the blood of the recipients was also similar, although the percentage of Pelger cells was much higher.

We believe that this method for the identification of transplanted bone marrow cells may be useful in experiments on rabbits, making it possible to follow the course of cytological changes during the recovery period in the bone marrow and peripheral blood as well. Moreover, this method may be applied for the simultaneous identification of all three donor bone marrow cell series and is more convenient than former methods.

We wish to express our thanks to Dr. E. Undritz, of the Sandoz Laboratories, for kindly supplying Pelger rabbits.

P. Czerski
O. Rosiek
J. Sabliński

Institute of Nuclear Research,
Department of Health Protection,
Dorodna 16, Warsaw.

[1] Congdon, C. C., in "Progress in Haematology", edit. by Tocantins, L. M., 2 (Grune and Stratton, New York, 1959).
[2] Undritz, E., *Folia Haematol.*, **67**, 249 (1943).
[3] Porter, K. A., *Brit. J. Exp. Path.*, **38**, 401 (1957).

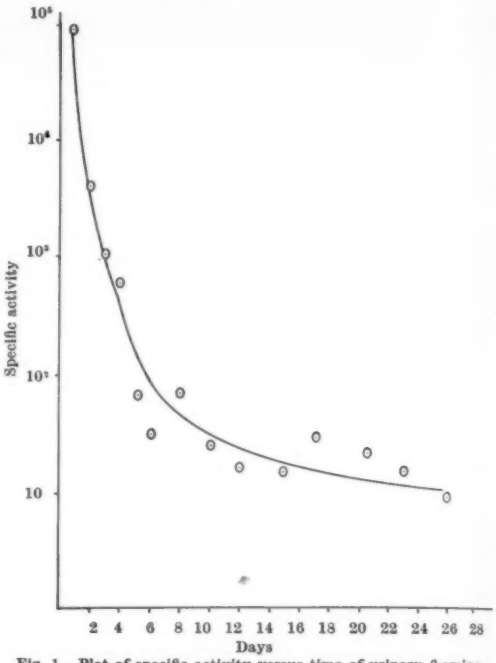


Fig. 1. Plot of specific activity versus time of urinary β-amino-*iso*butyric acid. The specific activity is expressed as the logarithm of c.p.m./μmole and time in days

Urinary Excretion of β-Amino-*iso*butyric Acid after Injection of Thymidine labelled with Tritium

β-Amino-*iso*butyric acid is a metabolic degradation product of thymine[1,2], but its relation to the catabolism of endogenous deoxyribonucleic acid has remained uncertain. In the present communication we present results which elucidate the catabolic fate of that fraction of thymine originating from endogenous deoxyribonucleic acid and reflected in the urinary excretion of β-amino-*iso*butyric acid.

Each of five rats weighing 130 gm. was injected intraperitoneally with $4 \cdot 5 \times 10^7$ c.p.m. of ^{3}H-thymidine in three equal doses given at 3-hr. intervals. The ^{3}H-thymidine used was prepared as described previously[3]. Pooled urine samples were collected from these animals during successive 24-hr. intervals throughout the 30-day experimental period. The rats were killed at various times after the injection of ^{3}H-thymidine. Deoxyribonucleic acid was isolated from several organs, as described previously[3], and β-amino-*iso*butyric acid from the urine specimens. A new method was used to determine β-amino-*iso*butyric acid[4]. This involved the initial conversion of β-amino-*iso*butyric acid to its dinitrophenyl derivative and the quantitative chemical determination thereof on the basis of the optical density at 360 mμ. Radioactivity of this dinitrophenyl derivative was determined in the manner described previously[3] using a gas-flow counter. The error involved in the determination of the specific activity of urinary β-amino-*iso*butyric acid was of the order of 10 per cent.

A plot of the specific activity of β-amino-*iso*butyric acid versus time is presented in Fig. 1. As can be seen, the specific activity decreased rapidly during the initial three days but only gradually thereafter. Presumably, the marked initial decrease in the specific activity results mainly from the utilization of thymidine for biosynthesis of deoxyribonucleic acid or degradation of thymidine *via* dihydrothymine and β-amino-*iso*butyric acid. Since on the second day the specific activity of tissue deoxyribonucleic acid exceeds that of urinary β-amino-*iso*butyric acid it appears reasonable to assume that at this time the breakdown of deoxyribonucleic acid contributes significantly to the labelling of β-amino-*iso*butyric acid. The rapid replacement of labelled deoxyribonucleic acid of intestine and hæmatopoietic tissue is thought to cause the initial decrease in the specific activity of urine. In other organs, such as liver or muscle, replacement of deoxyribonucleic acid is slower and may contribute radioactivity to β-amino-*iso*butyric acid at the later time period. The specific activity of deoxyribonucleic acid isolated from several organs on the third day is cited here for the purpose of comparison with the specific activity of β-amino-*iso*butyric acid, namely, liver, 2,800, muscle, 3,300, intestine, 7,400, and spleen, 6,300. The specific activity of intestinal deoxyribonucleic acid 24 hr. after injection of thymidine was 34,000. All values represent c.p.m./μmole of thymidine.

Since ^{3}H-thymidine may undergo exchange reaction during the metabolic conversion to β-amino-*iso*butyric acid, the fraction of thymidine which participates in the synthesis of deoxyribonucleic acid cannot be assessed quantitatively, although this could be accomplished by using thymidine labelled suitably with carbon-14.

Two other radioactive compounds were detected in the urine of these rats. One of these has been identified tentatively as dihydrothymine and resembles β-amino-*iso*butyric acid with respect to its curve of specific activity versus time. The second compound has not been identified as yet.

This work was supported, in part, by the United States Atomic Energy Commission.

GEORG B. GERBER
GISELA GERBER
KURT I. ALTMAN

Division of Experimental Radiology,
University of Rochester
School of Medicine and Dentistry,
Rochester, New York.

[1] Crumpler, H. R., Dent, C. E., Harris, H., and Westall, R. G., *Nature* **167**, 307 (1951).
[2] Fink, K., Henderson, R. B., and Fink, R. M., *Proc. Soc. Exp. Biol. Med.*, **78**, 135 (1951).
[3] Gerber, G. B., Gerber, G., and Altman, K. I., *J. Biol. Chem.*, **235**, 1433 (1960).
[4] Gerber, G. B., Gerber, G., and Altman, K. I., *Clinica Chemica Acta*, **5**, 607 (1960).

BIOLOGY

Electrical Sensitivity of the Ampullæ of Lorenzini

ELECTROPHYSIOLOGICAL investigation has shown that the ampullæ of Lorenzini of elasmobranch fishes are sensitive both to slight changes of temperature[1] and also to weak mechanical stimuli[2]. But neither sensory modality is convincing as the biologically adequate stimulus, for the temperature-sensitive regions are buried deep in the body (there being no apparent reason for the great anatomical development of the tube system), and the mechanical sensitivity is quantitatively less than that of the lateral line without being qualitatively very distinct.

There is, however, a third possibility. I have found that in rays the ampullæ are sensitive to slight changes in the electrical potential field surrounding the fish (*Raia clavata*, *R. naevus*, *R. montagui* and *R. brachyura* were used). The impulse discharge from single units in the mandibular group of ampullæ was recorded with the fish submerged about 1 cm. in a large dish of sea water; voltage gradients in the water over the sense organs were established from electrodes near the tail. Under such conditions the threshold d.c. potential gradient (that is, which produced approximately 10 per cent change of impulse frequency at make and break) was 1–2 μV./cm. (8 fish). The gradient had to lie along the line of the tubes, with the opening of the tubes negative to the capsule for an increase in frequency, and the opening positive for decrease. In each case there were opposite after-effects at break. The adaptation was almost complete, with a time-constant of about 5 sec. as is found with direct galvanic polarization of the sensory nerves[3].

With the preparation out of water, the ampullæ could be stimulated by passing a current between a small test probe and the body of the fish. When the probe was placed exactly on the opening of the tube from which the single fibre of the recording ran, a current of 0·005 μamp. (the smallest which could at the time be measured) was considerably greater than threshold; the frequency was increased when the probe was cathodal. But if the probe was displaced slightly from the opening, for example, by 0·5 mm., the threshold became far higher. Thus the stimulating currents would appear to be channelled along the jelly-filled tubes. In this connexion the conductivity of the jelly has been shown (Murray, R. W., and Potts, W. T. W., unpublished work) to be high (85–90 per cent of sea water) compared with that of the body fluids (40–45 per cent of sea water) and presumably the tissues have an even lower conductivity.

There would seem to be at least three possible ways in which this electrical sensitivity could be of direct use to the fish.

(1) It is known that rays have small electric organs derived from part of the tail musculature[4] which produce second-long pulses of up to 4 V. The potential gradients resulting from these would be at least 1,000 times stronger than those which I have found to be threshold for the ampullæ. Lissmann's suggestion[5] is therefore feasible, that this system could act as a navigation device, with the ampullæ detecting the changes in the field produced by the electric organs when an object with a conductivity different from the sea water comes near the fish. The ampullæ could of course act as the receptors for such a navigational system only in elasmobranchs, as they are not found in the teleosts with electric organs.

(2) I have some evidence that the ampullæ are sensitive to changes in the salinity of the sea water at their openings of about 5 per cent. The mechanism for this sensitivity could be electrical, depending on the differences in the diffusion potentials caused by dilution.

(3) When a conductor is moved through the Earth's magnetic field, potential gradients are set up in it. Under certain conditions it is possible to detect by this means the strength and direction of a tidal stream or ocean current. It is, however, theoretically improbable that a fish being carried along by the water movement, and therefore subject to the same influences, could detect these small potentials, although it might be able to determine whether the current was, for example, easterly or westerly by swimming up from a position at rest on the bottom.

R. W. MURRAY

Department of Zoology
and Comparative Physiology,
University of Birmingham.

[1] Sand, A., *Proc. Roy. Soc.*, B, **125**, 524 (1938).
[2] Murray, R. W., *J. Exp. Biol.*, **37**, 417 (1960).
[3] Murray, R. W., *J. Physiol.*, **145**, 1 (1959).
[4] Fessard, A., edit. by Grassé, P.-P., "Traité de Zoologie", **13**, 1143 (1958).
[5] Lissmann, H. W., *J. Exp. Biol.*, **35**, 156 (1958).

Development of Delayed-type Hypersensitivity in Guinea Pig Embryos

SEVERAL modalities of the immune response of embryos have been previously investigated. These include: (*a*) induction of immunological tolerance[1]; (*b*) antibody formation[2]; (*c*) capacity to reject homografts[3]. There have been no analogous studies of the capacity of the embryo to develop the delayed type of hypersensitivity to protein antigens.

Previous attempts to induce tuberculin skin reactivity in new-born guinea pigs by active sensitization[4] or by passive transfer with leucocytes[5] have been unsuccessful. Weiss[6] injected guinea pig embryos with tubercle bacilli, and elicited tuberculin reactions in some of these animals several weeks after birth.

In the present work, guinea pig embryos were injected *in utero* usually 1–2 weeks before the expected date of their delivery. In early experiments, the injections were made after laparotomy. Afterwards, injections were performed through the intact abdominal wall, using a 23-gauge needle directed at the position of the peritoneal cavity of the embryo, which was held between the fingers of the operator's other hand. In this fashion, 1–10 μgm. of protein

antigen contained in 0·1 ml. of complete Freund's adjuvant (including killed *Mycobacteria butyricum*) were injected into the embryo. Only two embryos in each litter were injected, since more injections greatly increased the rate of abortion. After birth, attempts to identify those embryos which had been injected were unsuccessful, despite the use in several experiments of proteins trace-labelled with iodine-131, and the inclusion of Evans blue dye in the suspension. This failure was probably due in part to the small amount of antigen injected and the slow release of the injected material from the saline-in-oil suspension. New-born guinea pigs were usually skin-tested on the first day of life with 3 μgm. of specific antigen. If the sensitizing injection had been administered less than five days before delivery, the skin testing was postponed until five days after sensitization.

Delayed-type reactions to diphtheria toxoid, ovalbumin, or bovine serum albumin similar to those previously described in adult animals[7] were elicited in 31 new-borns during the first five days of life. The average diameter of the reactions varied from 11 to 23 mm. Since a total of 83 injections were attempted in the embryos that survived, the sensitive animals probably represent a minority of those injected. Four skin reactions at 18–24 hr. and one reaction at 48 hr. after intradermal challenge were biopsied. Histological examination revealed pronounced infiltrations of lymphocytes, histiocytes and a smaller number of polymorphonuclear leucocytes throughout the dermis and subcutaneous tissue with an absence of vasculo-necrotic lesions (Fig. 1). Such lesions are typical of 'tuberculin-type' skin reactions[8].

Almost all the delayed-type skin reactions were obtained in animals weighing more than 90 gm. at birth, although an equal number of new-borns weighed between 50 and 90 gm. Since the weight of new-born guinea pigs is mainly a function of their intrauterine age[9], the capacity of guinea pig embryos to become sensitized probably depends in part on their general maturation.

In order to determine whether lymphoid cells were available in the smaller animals to participate in inflammatory reactions, total and differential white cell counts were carried out on three new-born animals that weighed 70 gm. each, and again on these animals two weeks later when they weighed 132–145 gm. At age one day the average white count was 2,800 with 41 per cent lymphocytes, compared to 4,900 with 65 per cent lymphocytes two weeks later, suggesting that there are an adequate number of lymphocytes in the circulation of the smaller new-born animal.

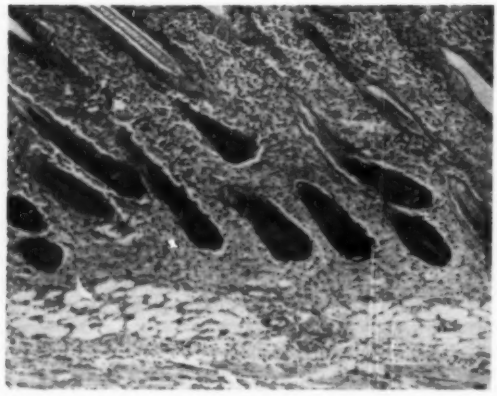


Fig. 1. Delayed-type skin reaction at 20 hr. elicited in a one-day old guinea pig. Hæmatoxylin and eosin. (× 45)

Table 1. Delayed-type Hypersensitivity in One-day Old Guinea Pigs previously sensitized *in utero*

Sensitizing antigen	Birth-weight (gm.)	Delayed skin reaction (mm.)	Passive cutaneous anaphylaxis test for serum antibody	
			Negative on day	Positive on day
Diphtheria toxoid	104	17 × 18	5	11
	104	11 × 12	11	—*
	94	22 × 25	11	—
	110	13 × 10	—	13
	124	18 × 17	—	11
	112	15 × 15	—	9
	96	15 × 15	—	9
Bovine serum albumin	112	20 × 20	3	6
Ovalbumin	100	17 × 16	2	10
	96	20 × 16	3	8
	88	17 × 18	3	8
	113	23 × 24	3	6

* Not done.

To ensure that delayed-type hypersensitivity in new-born animals was not the result of maternal sensitization and passive transfer across the placenta, twelve pregnant guinea pigs were injected intramuscularly 1–2 weeks before delivery with 1–10 μgm. of ovalbumin or toxoid in complete adjuvant. The new-born animals were skin-tested after their weight reached 100 gm. and none of fifteen animals showed delayed-type reactions.

Table 1 shows the positive skin reactions obtained in one-day old new-born animals in which antibody results using passive cutaneous anaphylaxis were also available. As seen in this table, antibody can be demonstrated in the serum of sensitive animals as early as one week after birth. When diphtheria toxoid was used as the antigen, the same serum in which antibody was demonstrated by passive cutaneous anaphylaxis failed to reveal antitoxin by the neutralization test for diphtheria toxin in rabbit skin. This is probably due to the production of antitoxin of low avidity, rather than to differences in sensitivity or specificity of the two tests. The possibility that antibody was produced against only non-toxoid diphtherial proteins is unlikely, since bovine serum albumin, which is less antigenic than toxoid for guinea pigs, stimulated detectable antibody formation.

To see if all new-born guinea pigs could be sensitized, twelve one-day old animals were injected either intramuscularly or in all four footpads with 1–10 μgm. of ovalbumin in complete Freund's adjuvant. Skin testing with 3 μgm. of ovalbumin 7–10 days later revealed delayed-type reactions in all those immunized in the footpads, but in only three of six immunized intramuscularly. Since the adult animal can be consistently made to acquire delayed-type hypersensitivity by the intramuscular route (if mycobacteria are included) it is possible that the new-born animal is sensitized less readily.

The capacity of one-day old animals to exhibit other hypersensitivity reactions was investigated, because of previous reports of the 'non-reactivity' of the skin of new-born guinea pigs[10]. To sensitize for passive cutaneous anaphylaxis reactions, 1 μgm. of rabbit anti-ovalbumin was injected intradermally into each of two new-born animals. Five hr. later, 1 mgm. of ovalbumin and Evans blue dye were injected intravenously. Blue reactions measuring 10 mm. by 10 mm. and 19 mm. by 17 mm. were obtained, but they were less intense than those simultaneously induced in adult

animals. To produce passive Arthus reactions, two new-born guinea pigs were injected intravenously, and two intraperitoneally, with 700 μgm. rabbit anti-ovalbumin followed 24 hr. later by intradermal injection of 30 μgm. ovalbumin. The intravenously sensitized animals developed typical Arthus reactions with maximal intensity at 4 hr., at which time the lesions averaged 45 mm. in diameter with a central hæmorrhagic area measuring 8–10 mm. in diameter. By 24 hr. the lesions had almost completely regressed. The animals sensitized intraperitoneally displayed milder reactions. Large necrotic lesions were also produced in neonatal animals by the intradermal injection of 0·025 MLD. of diphtheria toxin.

Our finding that delayed-type hypersensitivity can be induced in some guinea pig embryos is consistent with the relative maturity of this species at birth.

This work was aided by grants from the National Institutes of Health (*E* 1821 (*C*-2)) and the Commission on Immunization of the Armed Forces Epidemiological Board.

JONATHAN W. UHR

Departments of Microbiology and Medicine,
New York University School of Medicine,
New York.

[1] Billingham, R. E., Brent, L., and Medawar, P. B., *Nature*, **172**, 603 (1953).
[2] Fennestad, K. L., and Borg-Peterson, C., *Nature*, **180**, 1210 (1957). Sterzl, J., Kostka, J., Riha, I., and Mandel, L., *Folia Microbiologica*, **5**, 29 (1960).
[3] Schinkel, P. G., and Ferguson, K. A., *Austral. J. Biol. Sci.*, **6**, 533 (1953).
[4] Freund, J., *J. Immunol.*, **13**, 285 (1927).
[5] Waksman, B. H., and Matoltsy, M., *J. Immunol.*, **81**, 235 (1958).
[6] Weiss, D. W., *J. Exp. Med.*, **108**, 83 (1958).
[7] Uhr, J. W., Salvin, S., and Pappenheimer, jun., A. M., *J. Exp. Med.*, **105**, 11 (1957).
[8] Gell, P. G. H., and Hinde, I. T., *Brit. J. Exp. Path.*, **32**, 516 (1951).
[9] Ibsen, H. L., *J. Exp. Zool.*, **51**, 51 (1928). Draper, R. L., *Anat. Rec.*, **18**, 369 (1920).
[10] Freund, J., *J. Immunol.*, **17**, 465 (1929).

Insects attacking *Striga*

WORKING in Ibadan, Nigeria, Williams and Caswell[1] have recently noted *Smicronyx* sp. (Curculionidae : Coleoptera), the larva of which lives within the fruits feeding on the immature seeds of *Striga* sp. The only other reference mentioned is that of Agarwala and Naqvi[2] on *Striga* in Bihar, India. Williams and Caswell have apparently missed references on the subject, especially that on *Smicronyx albovariegatus* Faust. recorded on *Striga*.

The first published record of an insect attacking *Striga* was that of Murthy and Rao[3], who observed the caterpillars of *Precis orithya* Swinhoeii (Fam. Nymphalidæ, Lepidoptera) feeding on leaves, flower buds and tender fruits of *Striga* in the former Hyderabad State, India. Following the publication of that observation, Uttaman[4] pointed out that during the course of work on *Striga* in Malabar, India, he had noted grubs of a beetle (Sub-fam. Galerucinae) feeding on stem, leaves and the pods. While continuing work on the insect fauna of this root parasite in Hyderabad, I collected *Striga* stem galls in numbers caused by a curculionid weevil grub, and Mani[5], on the basis of the plant material sent from Hyderabad, described briefly the structure of the gall. Work was later initiated on the above insect at the Entomological Laboratory, Government Agriculture Research Station, Rudroor, Hyderabad State, and the weevil was identified as *Smicronyx albovariegatus* Fst. by the Commonwealth Institute of Entomology, London.

A brief account of the above insect was afterwards given by Khan and Murthy[6]. The adult weevils appear early in September and galls were noticed from the middle of September until January. A single grub is found in each gall. The number of galls per plant may vary from 1 to 30. When full grown, the grub comes out of the gall and pupates in the soil. The incidence of the weevil varies from 40·7 to 62·0 per cent. The grub not only causes stem galls but also eats into the seed capsules. During later work in Mysore State, another mode of injury caused to *Striga* plants by this weevil was recorded, namely, the formation of root galls by the grub[7]. It was observed that on account of the presence of root-galls there is weakening of the *Striga* plant which may perhaps be due to the impaired absorption of nutrients due to root damage.

Other insects recorded on *Striga* in India are *Monolepta signata* Oliv. (Galerucinae ; Chrysomelidae), caterpillars of a *Pterophorid* moth eating into the seed capsules[6,8] and a species of aphid awaiting determination.

While working in the Entomological Division, Government Agriculture Research Station, Rudroor, a detailed investigation was carried out on the possibilities of biological control of *Striga*, which is to be published shortly. It has been found that both *P. orithya swinhoei* and *S. albovariegatus* do exercise certain natural checks on the growth and development of *Striga* in the field. While no natural enemies have been encountered against *S. albovariegatus*, I have recorded[9] the caterpillars of *P. orithya swinhoei* being attacked by the tachinid endoparasite, *Sturmia* (*S. Str.*) *flavohalterata* Bisch, the percentage incidence ranging from 2·5 to 52·5. The eggs are also occasionally attacked by *Trichogramma* sp.

I am grateful to Drs. M. Q. Khan, joint director of agriculture, Andhra Pradesh, and M. Puttarudriah, Government entomologist, Mysore Agriculture Department, India, for facilities and for guidance and encouragement given during the course of this work.

D. V. MURTHY*

Division of Entomology,
Department of Agriculture,
Seshadri Road,
Bangalore 1.

* Formerly working in the Division of Entomology, Department of Agriculture, Hyderabad State. Present address : Chesterford Park Research Station, nr. Saffron Walden, Essex.

[1] Williams, C. N., and Caswell, G. H., *Nature*, **184**, 1668 (1959).
[2] Agarwala, S. B. D., and Haider Naqvi, S. Z., *Proc. Bihar Acad. Agric. Sci.*, **2**, 121 (1953).
[3] Murthy, D. V., and Rao, A. S., *Curr. Sci.*, **9**, 342 (1949).
[4] Uttaman, P., *Curr. Sci.*, **9**, 445 (1949).
[5] Mani, M. S., *Agra Univ. J. Res.* (*Science*), **2**, 2, 246 (1953).
[6] Khan, M. Q., and Murthy, D. V., *Ind. J. Ent.*, **17**, 3 (1955).
[7] Murthy, D. V., *Curr. Sci.*, **28**, 502 (1959).
[8] Khan, M. Q., and Murthy, D. V., *Sci. and Cult.*, **22**, 3, 175 (1955).
[9] Murthy, D. V., *Curr. Sci.*, **28**, 256 (1959).

Age Determination of Seals

A WORD of warning is necessary when the number of cementum rings in the canine teeth of seals is used to determine the age of some species of Phocidae. As Mansfield and Fisher[1] have stated, they were able to count 18–20 rings in a tooth of a harbour seal (*P. vitulina* L.) of 19 years of age. For practical purposes this is good enough in the higher range of years, but the possible error becomes disproportionately large if applied to years 1–10; years which contain the critical years of sexual maturity both potential and actual.

Great care is needed in assessing the age of young seals, since the doubt inherent in the statement '18–20' is derived from real difficulties met with in counting the rings deposited in the earlier years of life, and in assessing the value of the 'last' ring.

Some years ago it became desirable to know the exact age of specimens of the grey seal (*Halichoerus grypus* Fab.). The rings in the dentine are not obvious, and in any event, as Laws[2] had shown, the teeth (canine as well as the others) rapidly become filled with dentine so that after the first few years no further deposition is possible. The rings of the cementum offered an alternative provided they were annual. Thanks to the kindness of Mr. Colin Matheson, of the National Museum of Wales, a longitudinal section was obtained in June 1956 of the canine tooth of a cow grey seal which died at the age of at least 26½ [3]. In this at least 25–26 rings could be counted, but the exact number was doubtful. However, the supposition that the rings were annual in origin was upheld. In November 1958 a cow grey seal, exactly six years old, died in the Zoological Gardens, Regent's Park, London, and a section of the canine tooth showed six clear and complete rings. Meanwhile, grey seals approximately 6½ months and 7½ months old had been obtained, marked on the Farne Islands in the previous November. These two showed clearly the deposition in part of the first annual ring of cementum. More recently, younger grey seals have been obtained which show that cementum is first deposited about 3–4 months after birth, somewhat unevenly in two bands on the root. These bands afterwards coalesce.

The primary cause of the difficulty in counting the rings is that the earlier ones are broad without sharply defined edges. The evidence so far suggests that these broader rings are deposited before sexual maturity. The fact that they are visible at all suggests that the semi-starvation during the moult is responsible. Thus the 'years' measured by cementum rings are 'years' following moult. If the moult is many months after the breeding period (birthday) it is possible to count only, say, five rings when the seal is in its sixth year of life. Thus the 'last' ring must be looked for very carefully and the degree of its deposition related to the period of moult, if known.

Sufficient material (118 specimens) has now been collected for the grey seal and a paper giving full details and relating age with sexual maturity is in preparation. Much of this work must be repeated if the technique is to be applied critically to other species of Phocidae.

H. R. Hewer

Dept. of Zoology,
Imperial College of Science
and Technology,
London, S.W.7.

[1] Mansfield, A. W., and Fisher, H. D., *Nature*, **186**, 92 (1960).
[2] Laws, R. M., Falkland Is. Depend., Survey, Sci. Rep., 2 (1953).
[3] Matheson, C., *Nature*, **166**, 73 (1950).

Abnormal Crimping in Merino and Polwarth Wools

An abnormal wool, more apparent on older sheep and known as 'doggy' wool, is a cause of considerable financial loss in some Merino and Polwarth flocks. Such wool is frequently lustrous and is characterized by reduced frequency of crimps, so that in extreme cases the fibres are almost straight and hair-like. Preliminary evidence is presented here that doggy wool is associated with hyperplasia and cyst formation of the outer root-sheaths of a considerable proportion of the follicle population of affected sheep.

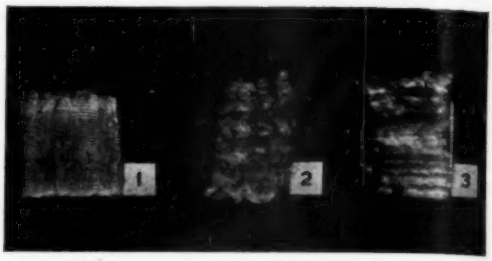


Fig. 1. Crimp deterioration in fine Merino and Polwarth wools in changing from normal (1) to 'doggy' (3)

In changing from normal to doggy, fine and medium Merino and Polwarth wools pass through an intermediate phase with secondary waves superimposed on the normal crimp; in strong Merino wool the change seems to be directly to a broader or irregular crimp. Fig. 1 illustrates the changes in fine Merino and Polwarth wools. The stages of crimp deterioration are somewhat similar to the changes produced in wool by a diet deficient in copper, but unlike the latter are not corrected by copper supplementation. The crimp aberrations are generally seen first in the loin, hip and thigh regions of the fleece, and the whole fleece may be affected within 1–2 years. The age at which the abnormality appears varies from 2 to 5 years in different strains of the Australian Merino, and the incidence also varies among the strains.

The main point of difference between normal and doggy wool is the changed crimp frequency. Routine examination of histological transverse sections of skin at the level of the sebaceous glands does not reveal any differences of significance. However, serial transverse and longitudinal sections of skin reveal grossly enlarged outer root-sheaths just above the bulbs of many follicles in the skin of sheep with crimp aberrations. Both primary and secondary follicles are involved, and in some follicles one or more localized enlargements form cysts, each of which is attached to the outer root-sheath by a narrow bridge of cells. Serial sections have established that these cysts are outgrowths from the outer root-sheaths. Fig. 2*a* shows a longitudinal section through a follicle with a cyst formed on an enlargement of the outer root-sheath, and Fig. 2*b* a transverse section of a number of follicles with outer root-sheath enlargements and cysts.

While occasional cystic sebaceous glands may be seen in the skin of sheep growing normal wool, particularly in aged sheep, the proportion of follicles with enlargements and cysts of the outer root-sheaths

Table 1. Percentages of Follicles showing Abnormalities in Mid-side Skin Samples from Sheep with Different Degrees of Crimp Aberration

Sheep	Crimping	Abnormal follicles (per cent)	
		With enlarged outer root-sheaths	With cysts
8- and 9-year old fine-wool Merinos	Normal	6	6
	Moderately doggy	34	7
	Very doggy	49	18
3 years old Polwarths	Well crimped	9	2
	Moderately irregular	25	8
	Very irregular	48	21

increased with severity of crimp deterioration (Table 1). Direct evidence of the association between enlargement of the outer root-sheath and reduction in crimping was obtained by plucking the straightest fibres from pieces of skin from sheep with different degrees of crimp aberration, and then sectioning the follicles longitudinally. In each instance of a follicle without a fibre there was enlargement of the outer root-sheath either with or without cysts.

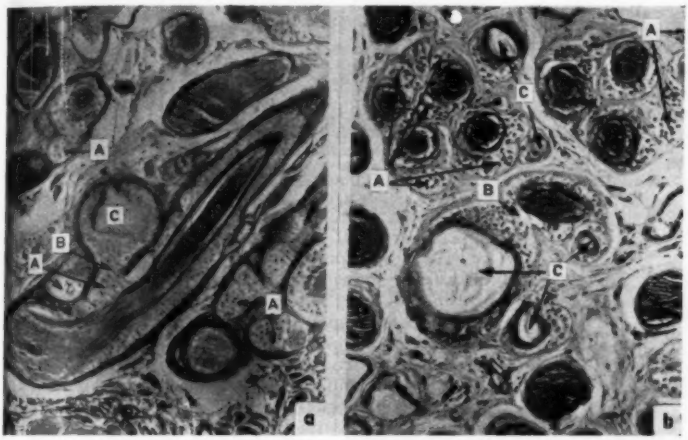


Fig. 2. *a*, A longitudinal section and *b* a transverse section through abnormal follicles showing enlargements of the outer root-sheaths (*A*) with cysts (*C*) attached by bridges of cells (*B*)

Although structures similar to these cysts have been described by others[1,2], to our knowledge the association between enlargement of the outer root-sheath and crimp deterioration has not been previously recorded. According to our evidence the outer root-sheath plays an important part in the process of fibre crimping. It may be, for example, that cyclic movement of the follicles is involved in the normal crimping of the fibres, and that these crimp irregularities are due to the added and variable resistance to movement of follicles with enlarged sheaths. The cysts could act as additional anchors, as they are frequently as large as the follicle bulbs and sometimes much larger. The observed proliferation of outer root-sheath cells is presumably accompanied by changed biochemical activity. This could conceivably alter the surface properties of the fibres, thus producing the lustrous appearance, and affect the process of crimping.

Abnormal crimping also occurs in fleeces of Corriedale sheep, and observations are being extended to this and other breeds. A detailed comparison is being made with the effects produced by a deficiency of copper in the diet, and this will be reported elsewhere.

R. E. Chapman
B. F. Short

Division of Animal Physiology,
Commonwealth Scientific and Industrial
Research Organization,
Prospect,
New South Wales.

P. G. Hyland

District Agricultural Centre,
Department of Agriculture,
Hamilton, Victoria.

[1] Ryder, M. L., and Auber, L., *Nature*, **174**, 743 (1954).
[2] Ryder, M. L., *J. Agric. Sci.*, **47**, 187 (1956).

Banana Fruit-spot ('Speckle') caused by *Deightoniella torulosa* (Syd.) Ell.

Deightoniella torulosa (Syd.) Ell. (syn. *Brachysporium torulosum* Syd.; *Cercospora musarum* Ashby; *Helminthosporium torulosum* (Syd.) Ashby) has been recorded on banana leaves and fruits in most major growing regions (Commonwealth Mycological Institute, Kew, Surrey, Distribution Map No. 175). The majority of earlier references to this fungus have concerned a leaf disease known as 'black-spot'[1-3] and a blackening of the tips of banana fruits[4,5].

A serious spotting of Lacatan bananas known in Jamaica as 'speckle' or 'swamp-spot' causes considerable wastage, particularly during rainy seasons. Fruit-spot may be observed in the field on fruit at all stages of maturity, the intensity of spotting increases with maturity being most noticeable at the time of harvest. In immature, green bananas, disease is first manifest as minute, reddish-brown spots each having a characteristic halo of dark green, apparently water-soaked tissue. As the spot increases in size, its outline remains circular, or nearly so. The largest spots observed so far had diameters of about 4 mm.: in these cases, the centre of the spot was dark brown or black and slightly depressed. The large proximal ('butt-end') hand of the bunch is usually more severely affected than the smaller, more distal ones. On each finger (that is, individual banana fruit), spots tend to be concentrated, on the outer face, towards the tip, and, on the inner face, towards the finger-stalk. There is evidence to suggest that the severity of spotting increases during ocean transportation and subsequent ripening of fruit.

External symptoms and anatomical details of the Jamaican fruit-spot are similar to those of a spot described by Johnston[6] and Johnston and Slocum[7] on Gros Michel bananas in Central and South America. These workers considered fruit-spot to be in the nature of a physiological disorder. Wardlaw[8] described several forms of spotting of bananas in Trinidad, but none of them would appear to be identical with Jamaican 'speckle'.

In the present investigation, *D. torulosa* was consistently isolated from spots of various diameters: no other fungus (or bacterium) appeared on the isolation plates. Pathogenicity tests were carried out in the laboratory and in the field by spraying the surface of apparently healthy fruit with a conidial suspension of *D. torulosa*. After 48 hr. under high humidity conditions, small but typical spots appeared on all inoculated fruit. Control fruit, sprayed with sterile water only, did not develop spots. *D. torulosa* was consistently re-isolated from artificially induced spots.

In a film of water on the surface of banana fruit peel, conidia of *D. torulosa* germinated after 1 hr.: after about 6 hr. one of, or both, the germ-tubes produce a swollen appressorium. Vertical microtome sections, 8–10μ thick and stained with 1 per cent cotton blue in lactophenol, showed that the appressorium soon produces an infection hypha which penetrates the cuticle and epidermal wall by mechanical means. This stage

of infection was observed after 12–20 hr. After penetration has occurred, the epidermal cell becomes filled with a reddish-brown, granular substance. Further tissue invasion occurs by hyphal penetration of cell walls.

Similar stages in this infection process were observed on naturally infected fruit.

From the evidence outlined above it seems clear that *D. torulosa* is the organism causing fruit-spot of Jamaican Lacatan bananas. A detailed account of this work will be published elsewhere.

D. S. Meredith

Banana Board Research Department,
10 South Avenue, Kingston, Jamaica.

April 18.

[1] Ashby, S. F., *Bull. Dep. Agric., Jamaica*, **2**, 95 (1913).
[2] Wardlaw, C. W., *Trop. Agric.* (Trin.), **11**, 13 (1934).
[3] Stahel, G., *Trop. Agric.* (Trin.), **11**, 138 (1934).
[4] Ogilvie, L., *Phytopath.*, **18**, 531 (1928).
[5] Wardlaw, C. W., *Trop. Agric.* (Trin.), **9**, 3 (1932).
[6] Johnston, J. R., Res. Dep. United Fruit Co., Boston, Mass., Bull. No. 43 (1932).
[7] Johnston, J. R., and Slocum, A. H., Res. Dep. United Fruit Co., Boston, Mass., Bull No. 41 (1932).
[8] Wardlaw, C. W., *Trop. Agric.* (Trin.), **11**, 8 (1934).

A Method for the Isolation of Plant Protoplasts and Vacuoles

The isolation of bacterial[1] and fungal protoplasts[2,3] following the use of enzymes digesting the cell wall suggested that protoplasts might be obtained from plant cells by treatment with cellulase. Root tips of tomato seedlings (*Lycopersicum esculentum* Mill. var. Sutton's 'Best of All') were selected for investigation, and a fungal cellulase obtained from *Myrothecium verrucaria*[4] (kindly supplied by Dr. D. R. Whitaker, National Research Council, Canada) was employed.

0·5-cm. root tips of tomato seedlings grown under aseptic conditions were treated at 27° C. with 5 per cent *Myrothecium* cellulase in 0·02 *M* phosphate buffer *p*H 6·0, containing 0·6 *M* sucrose as an osmotic stabilizer. Microscopic examination of the root tip, mounted under a cover slip, showed the formation of numerous spherical protoplasts after 2 hr. of treatment with cellulase. Two types of protoplast were identified. One type was unvacuolated (Fig. 1), and could be seen to be formed from the cells of the unvacuolated meristematic region of the root tip. The other type of protoplast was vacuolated with one or more vacuoles (Fig. 2) and was formed from vacuolated cells approximately 1 mm. from the root cap. Prior to their liberation these vacuolated protoplasts could be seen inside the remains of the partly digested cell walls, and it was evident that plasmolysis of cells by the hypertonic buffered sucrose was assisting in the ready isolation of intact vacuolated protoplasts.

These vacuolated protoplasts were stable in 0·3 *M* sucrose (0·02 *M* phosphate buffer, *p*H 6·0) for at least 6 hr. at 27° C. However, when suspended in 0·1 *M* sucrose (0·02 *M* phosphate buffer, *p*H 6·0) rapid lysis ensued with bursting of the plasmalemma and concomitant release of intact, yet swollen,

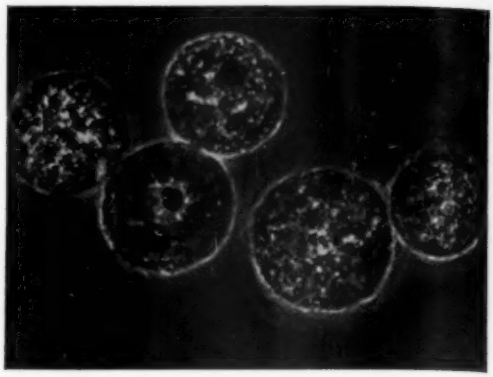


Fig. 1. Unvacuolated protoplasts (phase contrast)

vacuoles together with the protoplasmic contents. A typical group of such isolated vacuoles surrounded by their tonoplasts with adhering protoplasmic material is shown in Fig. 3. It was noteworthy that protoplasts were not formed from cells of the more differentiated region of the root tip bearing root hairs. The failure to isolate protoplasts from cells of this region was probably related to the different state of the cell wall[5] and to the presence of plasmodesmata linking the protoplasts of adjacent cells[6].

It is clear that the vacuolated structures shown in Fig. 2 are protoplasts as defined on the basis of the criteria of Brenner *et al.*[7], since they are structures formed from that part of the cell which lies inside the cell wall and which can be plasmolysed away from the cell wall. In the case of the unvacuolated structures, shown in Fig. 1, it is more difficult to establish on the basis of similar criteria that these are in fact protoplasts, since they are formed from meristematic cells which are not readily plasmolysed[8] in which the cell wall is very intimately associated with the protoplasm[5]. Nevertheless, it was possible to observe various stages in the removal of the cell wall of these unvacuolated cells before attainment of the final

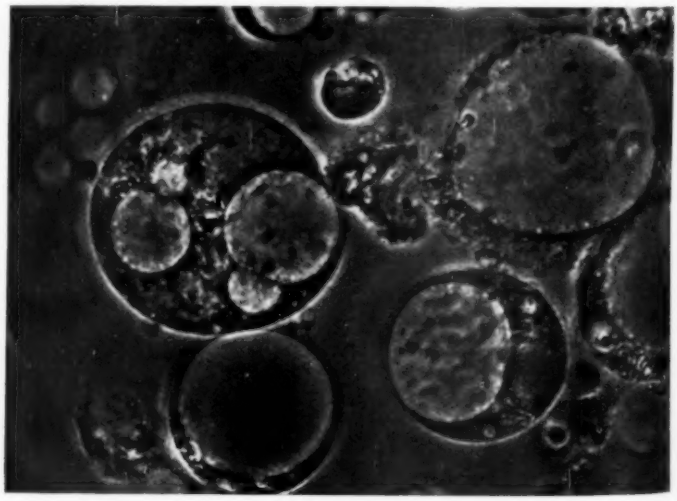


Fig. 2. Vacuolated protoplasts (phase contrast)

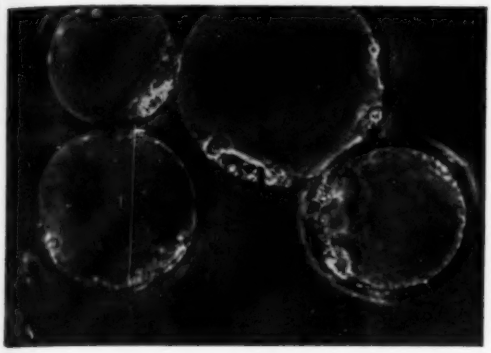


Fig. 3. Vacuoles (phase contrast)

spherical form, both in cellulase-treated root tips and in cellulase-treated single unvacuolated cells isolated from root tips by the action of ethylenediamine tetraacetic acid[9].

E. C. Cocking

Department of Botany,
University of Nottingham.

[1] Weibull, C., "Ann Rev. Microbiol.", **12**, 1 (1958).
[2] Eddy, A. A., and Williamson, D. H., *Nature*, **179**, 1252 (1957).
[3] Bachmann, B. J., and Bonner, D. M., *J. Bact.*, **78**, 550 (1959).
[4] Whitaker, D. R., *Arch. Biochem. Biophys.*, **43**, 253 (1953).
[5] Preston, R. D., "The Molecular Architecture of Plant Cell Walls" (Chapman and Hall, London, 1952).
[6] Mecuse, A. D. J., *Bot. Rev.*, **7**, 249 (1941).
[7] Brenner, S., Dark, F. A., Gerhardt, P., Jeynes, M. H., Kandler, O., Kellenberger, E., Klieneberger-Nobel, E., McQuillen, K., Rubio-Huertos, M., Salton, M. J. R., Strange, R. E., Tomcsik, J., and Weibull, C., *Nature*, **181**, 1713 (1958).
[8] Bennet-Clark, T. A., in "Plant Physiology", edit. by Steward, F. C., 2 (Academic Press, 1959).
[9] Cocking, E. C., *Biochem. J.* (in the press).

ENTOMOLOGY

Bacteria as a Source of Food for Black-Fly Larvæ

Black-fly larvæ (Diptera : Simuliidae) are almost entirely confined to flowing water. They have long been known as 'filter-feeders', but the minimum particle-size acceptable as food by the several instars has not been previously investigated. Several authors have reported the occurrence of larvæ in sewage-contaminated waters ; but Petersen[1] was apparently the first to suggest that they might feed on suspended particles as small as bacteria.

This hypothesis is under investigation at Saskatoon in an attempt to determine some of the causes of destructive outbreaks of the black-fly, *Simulium arcticum* Mall. Larvæ of this species are sometimes excessively abundant in the north and south branches of the Saskatchewan River which originate in the Rocky Mountains of western Canada and discharge up to 140,000 c.f.s. into the confluence. During the spring and early summer, when these larvæ are most abundant, this river is generally in flood and the water very muddy with up to 1,000 p.p.m. of suspended solids. At the same time, macroplankton, the usual food of black-fly larvæ, are relatively scarce but bacteria are abundant. Species of bacteria present indicate an origin in sewage from the several cities situated on the river as well as from soil erosion.

In preliminary experiments at Saskatoon an attempt was made to rear first-instar larvæ of *S. vittatum* Zett. and *S. venustum* Say in sterile solutions of soluble nutrient in water flowing at 0·3–1·4 ft. per sec. In the absence of discrete particles these larvæ failed to develop beyond the second instar[2]. In similar, but non-sterile, solutions the larvæ developed to maturity, and adults were produced. Five species of bacteria, including three commonly found in soil and water, were isolated by Dr. G. Dempster, University of Saskatchewan, Saskatoon.

In further experiments 90 adults of these two species of black-flies were reared from 1,360 first-instar larvæ in distilled water containing only a washed suspension of *Bacillus subtilis* (Ehr.) Cohn, a normal component of soil. Fresh bacteria were added at 2- to 3-day intervals, keeping populations at about 160,000 viable bacteria per ml. of aquarium water. Microscopic examination showed that the bacteria were not clumped, and that most were present as single cells rather than in chains. About 65 per cent of the larvæ were lost in the first instar, possibly as a result of injuries received in handling. Rate of development was comparable to that in Nature : 21 days from hatching to the first pupa and a further 6 days to the first adult. These investigations are continuing and a detailed report will be published later.

Laboratory experiments have thus far been confined to larvæ of *S. vittatum* and *S. venustum* because of the difficulty in providing rapidly flowing water as in aquaria required by *S. arcticum*. However, the mouth-parts of these three species are very much alike, and thus similar results with the latter species may eventually be obtained.

The fact that all instars of larvæ of *S. vittatum* and *S. venustum* can obtain adequate nutriment from particles as small as *B. subtilis* is significant from two points of view : (1) Sewage bacteria entering a river may serve as food for black-fly larvæ. Thus the severity of black-fly outbreaks originating in rivers such as the Saskatchewan, Mississippi, Danube, Leine, Nile, etc., may be expected to increase as the amount of sewage entering them is increased. Reports[3,4] and personal observations indicate that livestock losses as a result of black-fly outbreaks along the Saskatchewan River have increased in the past 40 years. Enigk[5] reports that black-flies are appearing in larger numbers in Germany, and suggests that this is due to increased soiling of brooks and rivers. Losses adjacent to the Danube River appear to have been increasing during the past two hundred years[6]. (2) This suggests an unusual food-chain in the Saskatchewan River and perhaps other rivers which seasonally contain relatively little macroplankton but considerable bacteria and numerous filter-feeding black-fly larvæ. Fish, including *Moxostoma aureolum* (Le Sueur) and *Hiodon alosoides* Raf., containing black-fly larvæ were collected from the Saskatchewan River. Numerous larvæ-eating fish are known from the Danube[6].

F. J. H. Fredeen

Research Station,
Canada Department of Agriculture,
Saskatoon, Saskatchewan.

[1] Petersen, Axel, *D. Kgl. Danske Vidensk. Selsk. Skrifter*, Afd. 8, R., **4** (1924).
[2] Fredeen, F. J. H., *Canadian Ent.*, **91**, 2, 73 (1959).
[3] Cameron, A. E., Canada Dept. Agric. (Tech.) Bull. 5 (n.s.) (1922).
[4] Rempel, J. G., and Arnason, A. P., *Sci. Agric.*, **27**, 428 (1947).
[5] Enigk, Karl, *Monatshefte für Tierh.*, **7**, 11, 241 (1955).
[6] Zivkovic, Vera, Serbian Acad. Sci., Special Issue, Book 245 (1955).

Accessory Glands of Gene's Organ in Ticks

During dissection of gorged adult females of *Hyalomma excavatum*, *Rhipicephalus sanguineus* s.str. and *Boophilus annulatus*, we noted two hitherto undescribed groups of accessory glands, one on each side of Gené's organ.

The glands become evident soon after the onset of feeding and continue to develop during the period of digestion of the blood meal. In *Ripicephalus*, which oviposits 2–3 days after gorging, the development is completed during this interval; in *Hyalomma*, in which oviposition occurs ten days after gorging, the development is correspondingly slow. The glands attain full development by the time oviposition begins. They were not detected in the starved female or the nymph. We did not find these glands in adult *Ornithodorus tholozani*.

Fig. 1. New accessory gland from a female of *Rhipicephalus sanguineus* s.str., on the third day of oviposition. (× 140)

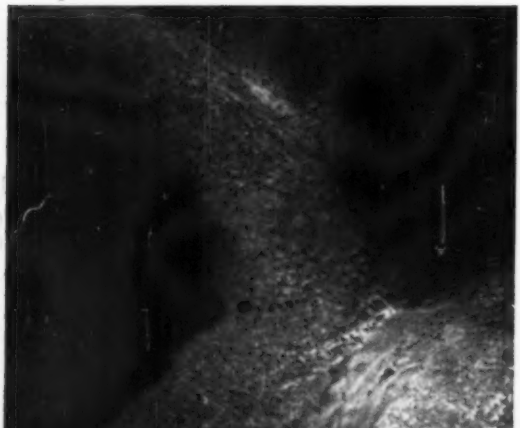


Fig. 2. Droplets of secretion on their way through the intracellular channel from a laying female of *Rhipicephalus sanguineus* s.str. (× 660)

Each group of glands consists of about 40 units in the case of *Hyalomma* and between 20 and 25 in the case of *Rhipicephalus*.

Each gland consists of about 10–13 cells. In general the cells are arranged roughly in a circle (Fig. 1). The large nucleus (up to 85μ) is situated in the lateral part of the cell; it contains several nucleoli. In each cell there is a large vacuole which grows continuously and attains a size of about 100–130μ at its maximum development. Each gland has a duct issuing from its centre; a narrow intra-cellular channel passes from each vacuole to the narrow main duct. On staining with methylene blue, droplets can be traced on their way from the vacuole via the narrow intra-cellular channel into the duct (Fig. 2).

These glands apparently contribute to the secretions of Gené's organ which protect the eggs in their external environment.

This investigation was supported by Research Grant No. 4531 from the National Institutes of Health, U.S. Public Health Service.

B. Feldman-Muhsam
Y. Havivi

Department of Parasitology,
The Hebrew University,
Jerusalem.

CYTOLOGY

Induced Fluorescence with Acridine Orange, after Osmium Fixation

The differential staining of nucleic acids with fluorochromes[1] has provided a useful tool for the study of virus-infected tissue cells[2]; but its value has been limited by the fact that the common fixatives which permit the use of this method also tend to produce artefacts, such as the gross nuclear vesiculation observed in cells infected with some types of adeno-virus[3], but not observed by phase-contrast examination of the living infected cell[3].

This serious limitation could be overcome if the technique could be adapted for use with fixation by osmium tetroxide, which has been shown to preserve faithfully most of the structural features of the living cell[4,5]. Up to the present, however, this has not proved possible, and reports indicate practically no affinity between the diamino-acridines and nucleic acids fixed in osmium tetroxide[3].

Recent work in this laboratory has shown that this lack of affinity may be overcome if staining is carried out with acridine orange in alcoholic solution, in place of the buffered aqueous solution, as usually practised. The technique described is simple and uniformly successful, giving vivid colour differentiation and even staining over the entire preparation. Acridine orange *R* (2,8-*bis*-dimethylamino-5-phenyl-acridine hydrochloride, Colour Index No. 792) in 1 : 1,000 dilution in industrial methylated spirit ('Methcol') was used, and this was filtered before use. The end result was found to be the same, however, using dilutions of lesser or greater strength, and allowing for an appropriate alteration in the staining time.

Cells of the 'ERK' cell line[6] were grown on glass microscope slides to form a near-confluent monolayer. The medium was washed off with phosphate-buffered saline, *p*H 7·2, and sample slides were then fixed either in the vapour from 1 per cent osmium tetroxide

for 10 min., or in the buffered osmium tetroxide solution of Palade[5] for the same length of time. Both sets of cultures were thoroughly washed in several changes of distilled water, during half an hour, and this was followed by a brief rinse in 'Methcol'. They were then transferred to a covered staining jar containing the alcoholic solution of acridine orange, and stained for 10 min. On removal, the slides were thoroughly washed in phosphate buffer made up of potassium dihydrogen phosphate 4·5 gm., ammonium chloride 0·5 gm., ammonium sulphate 0·5 gm., per litre adjusted to *p*H 7·6 with sodium hydroxide and finally mounted in liquid paraffin.

Examination was carried out with a simple system using a projector lamp of the solid-source filament type, an Abbé type condenser, a cupro-ammonium cell of suitable density, and a yellow eye-piece filter. An alternative system, using a high-discharge mercury vapour lamp, dark ground condenser, and appropriate filters, did not materially improve the picture obtained.

Both methods of fixation gave equally good results, with sharp differentiation of the cellular elements corresponding to the nucleic acid pattern. The nucleoplasm of interphasic cells appeared homogeneous, fluorescing as a clear, bright greenish-yellow. Nucleoli showed prominently as discrete, red bodies with their surrounding zone of 'associated' deoxyribonucleic acid seen as a halo of more brightly fluorescing yellow. The ribonucleic acid of the cytoplasm was uniformly intense, deep red. In dividing cells, mitoses were distinctive by their brilliant golden fluorescence and sharp configuration.

Semi-permanent preparations of this type, allowed to remain on the bench at room temperature for at least a month after sealing, have shown no deterioration. Liquid paraffin was chosen as the mountant, in preference to either glycerine, or glycerine–saline, since the fluorochrome is apparently unaffected by it, and no extraction of the dye occurs even after prolonged contact. Finally, it is a simple matter to renew bleached preparations, by removing the liquid paraffin with washes of alcohol and then repeating the treatment with alcoholic acridine orange.

W. J. Randles

Microbiological Research Establishment,
Porton,
Wilts.

[1] Armstrong, J. A., *Exp. Cell Res.*, **11**, 640 (1956).
[2] Anderson, E. S., Armstrong, J. A., and Niven, J. S. F., Ninth Symp. Soc. Gen. Microbiol., 224 (1959).
[3] Armstrong, J. A., and Hopper, P. K., *Exp. Cell Res.*, **16**, 584 (1959).
[4] Porter, K. R., Claude, A., and Fullam, E. F., *J. Exp. Med.*, **81**, 233 (1945).
[5] Palade, G. E., *J. Exp. Med.*, **95**, 285 (1952).
[6] Westwood, J. C. N., MacPherson, I. A., and Titmuss, D. H. J., *Brit. J. Exp. Path.*, **38**, 138 (1957).

A Polyploidizing Principle in the Spleen

In previous experiments it has been found that 120 hr. after traumatization of the uterus the number of large homogeneous cells in the antimesometrial part of the deciduomata was doubled in splenectomized rats[1]. In other experiments the animals were partially omentectomized or unilaterally nephrectomized. But there was no difference in cell count between these animals and the non-splenectomized controls[2]. The proportion of the volume of the nuclei of the large decidua cells between normal rats and spleenless ones[3] was approximately 2 : 1. The number of countable nucleoli in each nucleus shows the same ratio[4].

These changes, appearing after the extirpation of the spleen, can be prevented by saline extracts of the spleen from rat, cattle and horse. Further, it has been shown that a linear relation exists between the logarithms of the quantity of injected saline extract and the number of large decidua cell nuclei. From these experiments one may conclude that the discernible changes in the nuclei after splenectomy were dependent on changes of the degree of ploidy. Therefore a substance producing a polyploidizing effect was supposed to exist in the spleen[1]. The direct evidence of that polyploidizing effect was obtained by counting the chromosomes.

With female inbred rats of an average body-weight of 200 gm. a pseudo-pregnancy was induced by sterile copulation. 'Spirocid' (167 mgm./kgm. body-weight/day) was then given orally for three days to prevent the manifestation of a latent infection with *Bartonella muris ratti* after splenectomy. 96 hr. after the sterile copulation the animals were operated under ether anæsthesia : both horns of the uterus were traumatized by making several stitches through the lumina. In six animals the spleens were removed in addition ; in three animals serving as controls parts of the omentum were excised. The splenectomized animals were divided into two groups. One group remained untreated. The other group was subcutaneously injected with saline extract of cattle spleen immediately after the operation and for the following four days. 120 hr. after operation all animals received 0·2 mgm./100 gm. body-weight colchicine intracardially and were killed by carbon monoxide 2 hr. later. Then the

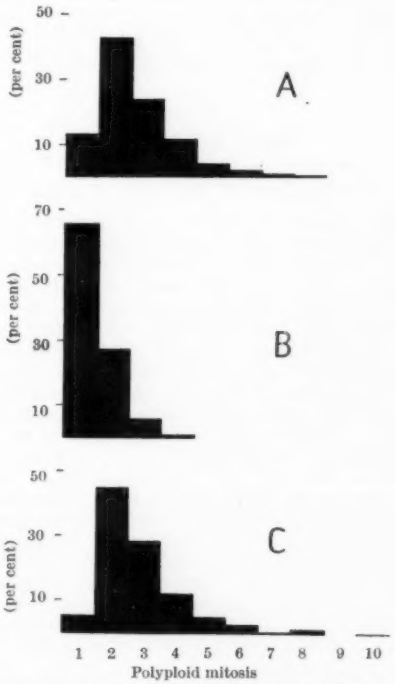


Fig. 1. Three histograms demonstrating the distribution of the degree of ploidy in metaphase plates of the large decidua cells. Each histogram is based on 500 mitoses of three rats. *A*, omentectomized rats ; *B*, splenectomized rats ; *C*, splenectomized rats which received saline extract of cattle spleens

uteri were prepared, the myometrium was dissected and the antimesometrial decidua was cut out. After removing the adhering blood the tissue was cut into small pieces which were suspended in 0·00075 *M* 8-oxychinoline solution for 1 hr. The tissue was then fixed for 3 hr. in ethanol–acetic acid, 3 : 1, and afterwards preserved in 70 per cent ethanol. For further preparation these pieces of tissue were stained for 30 min. in carmine–acetic acid containing 10 per cent glycerol[5]. Then they were picked with needles into tiny pieces and squashed under a cover-glass. The chromosomes were counted by means of a phase-contrast microscope at 2,000- or 3,000-fold magnification. Only metaphase plates were examined. Each mitosis was counted independently by two persons. In each group the chromosomes in 500 mitoses were counted. The numbers we found were classified as to the degree of ploidy. The haploid set of chromosomes was : $n = 21$. The limits of these degrees of ploidy were fixed as : $n \pm 10$, $2n \pm 10$, $3n \pm 10$ . . . . In total, 1,500 metaphases were counted in the antimesometrial part of the rat deciduomata. The results of these counts are demonstrated in Fig. 1.

From this it may be concluded that in the spleen there exists a polyploidizing substance, the chemical nature of which is not yet known.

I. Bouvier
R. Rigler
W. Rosenkranz

Institute of Physiology,
University of Graz,
Harrachgasse 21,
Graz.

[1] Rigler, R., and Rosenkranz, W., *Z. Naturforsch.* **13**, b, 291 (1958).
[2] Rigler, R., and Rosenkranz, W., *Wien. klin. Wschr.*, **69**, 445 (1957).
[3] Rosenkranz, W., and Rigler, R., *Z. ges. exp. Med.*, **129**, 627 (1958).
[4] Rigler, R., and Rosenkranz, W., *Z. Naturforsch.*, **13**, b, 543 (1958).
[5] Marquardt, H., and Gläss, E., *Chromosoma*, **8**, 617 (1957).

SOIL SCIENCE

C Horizon of the Soil Profile

In the course of recent investigations on the pedology of fluvial soils located in the area of Durham City, no true *C* horizon could be observed in the numerous profiles and sections examined. In the case of so-called transported soils, it is, of course, well known that the *D* horizon will usually be unrelated to the solum. But the finding that the *C* horizon is absent in some of these soils is of pedological importance, and, so far as we are aware, has not previously been reported for soils of the United Kingdom. Nevertheless, the occurrence of soil profiles of this type would seem to be of more than local significance and may well be widespread.

The area under study formed part of the floor of a large glacial lake, which came into existence towards the end of the Pleistocene epoch, when varying thicknesses of fluvioglacial deposits were laid down. These deposits, which are often lenticular, are both heterogeneous and variable, particularly in texture, structure, colour, depth and drainage; they show clear evidence of water-sorting in the morphology of the profiles and sections.

The alluvial soils studied are located in an old flood plain of the River Wear and thus represent the results of the resorting and deposition by the action of water on the glacial and fluvioglacial deposits left after the retreat of the ice. They are therefore of similar mineral content to the local fluvioglacial deposits. These deposits are also extremely heterogeneous.

In general, the study of the profiles and sections of both fluvioglacial and alluvial soils revealed two distinct horizons—a zone of eluviation and a zone of illuviation; but it was not possible to distinguish a true *C* horizon although the investigation extended to Carboniferous age bedrock (shale) encountered between 19 and 24 ft. The *C* horizon is by definition the unconsolidated parent material substantially similar to that from which the solum has developed. Before such a horizon can be identified there must be evidence of this similarity, and this is not apparent in the profiles and sections studied. In these soils the parent material of the upper layer of the soil profile has been completely transformed into the *A* horizon. Probably it was different from the parent material of the *B* horizon and was not necessarily identical with any mineral material that can now be observed in the regolith. The relationship between the different soil horizons is dependent on pedological processes acting on parent material horizontally bedded and initially heterogeneous. The horizons below the zone of illuviation must be regarded as *D* horizons, because any unconsolidated material beneath the solum not characterized as parent material is so designated[1]. In these soils the succession of horizons is therefore *A*,*B*,*D*.

It will be seen that the absence of a *C* horizon is, in effect, the result of one or more relatively thin layers of transported material originally constituting the upper zone of the deposits. A parallel case of interest has been recorded in the United States in regard to certain deposits of loess only 30 in. deep[2]. It is known that the *C* horizon may also be absent in the profile of soils developed *in situ* where the parent material is sufficiently resistant to weathering to result in the rate of formation of the solum keeping pace with the rate of rock weathering.

A further relevant experience has recently been encountered in an investigation of types of rendzina profiles developed on the magnesian limestone of Co. Durham. Glacial till was found to overlie the bedrock in some of the profiles studied. In cases where the layer of till was thin the typical rendzina profile was developed, but the *A* horizon has been formed from varying proportions of both till and the residues remaining from the weathering of magnesian limestone. In many cases the effective contribution of till is sufficiently great to preclude the underlying weathering magnesian limestone from being classified as parent material. In the case of these soils, the succession of horizons is therefore *A*,*D*.

It is hoped shortly to present elsewhere detailed reports of all these investigations.

S. G. Willimott

Department of Geography,
The Durham Colleges in the
University of Durham,
South Road,
Durham.

D. W. Gilchrist Shirlaw

Durham School of Agriculture,
Houghall,
Co. Durham.

[1] Lyon, T. L., Buckman, H. O., and Brady, N. C., "The Nature and Properties of Soils", fifth ed., 327 (Macmillan Co., New York, 1952).
[2] Thompson, L. M., "Soils and Soil Fertility", second ed., 127 (McGraw-Hill, New York, Toronto and London, 1957).

Effect of Surface Density of Charge on the Physical Swelling of Lithium Montmorillonite

THE crystalline swelling of lithium montmorillonite before and after heating has been investigated by several workers[1-3]. Hofmann and Klemen[1] suggested that, on heating, the small lithium ion migrated into the crystal lattice to occupy a vacant octahedral site with consequent neutralization of the charge originating in that layer due to the isomorphous replacement of Al^{3+} by Mg^{2+}. They termed this 'place exchange'. This process gives rise to marked reduction in crystalline swelling.

Greene-Kelly[3] has followed by an X-ray technique the loss in glycerol adsorption for Li^{+} saturated minerals over the range of composition from montmorillonite to beidellite. For less than 70 per cent octahedral substitution he has found some penetration of glycerol. Complete penetration was obtained at about 10 per cent octahedral substitution. In other experiments he studied the effect of only partly saturating the exchange complex of montmorillonite with lithium; the other ions balancing the negative charge were normal in their behaviour after dehydration. At about 44 per cent saturation with lithium complete penetration of glycerol was observed, but for 68 per cent saturation with lithium only about one quarter of the internal surface was accessible to glycerol.

Recently, Johns and Tettenhorst[4] have demonstrated that the order of penetration into dehydrated crystals is ethylene glycol > glycerol > water. It was thought that a study of the physical swelling of Wyoming bentonite containing varying proportions of lithium and sodium might give useful information on the minimum surface density of charge required for large intracrystalline expansion.

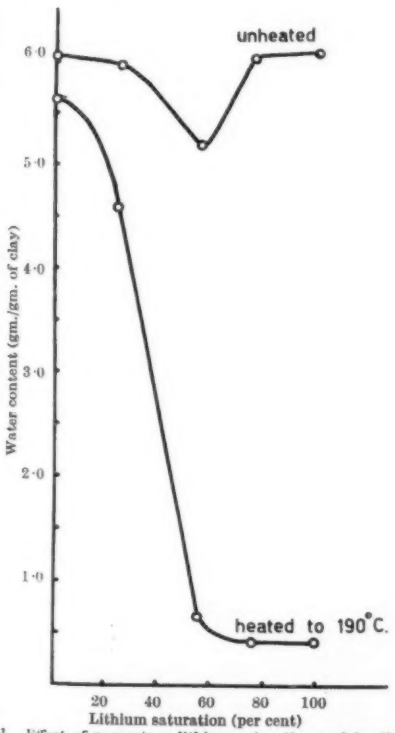


Fig. 1. Effect of percentage lithium saturation and heating on the water content of clay cores at 100 cm. of water suction

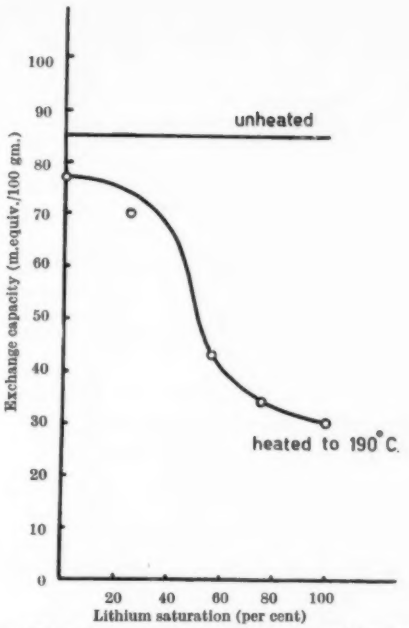


Fig. 2. Effect of percentage lithium saturation and heating on the exchange capacity of montmorillonite

Compressed cores of the clays were manufactured[5,6] from the control and heated clay powders. These cores were allowed to swell slowly to equilibrium with a hydrostatic suction of 100 cm. of water. The wetting liquid was distilled water and the results plotted in Fig. 1 reveal that at 56 per cent lithium saturation the clay has lost its capacity for large intracrystalline swelling as the control water content is greater than 5 c.c./gm. and the heated clay has a water content of 0·62 c.c./gm. This latter result can be compared with that for sodium montmorillonite in the presence of *M* sodium chloride when the solution content was found to be 0·90 c.c./gm.; for these circumstances the intracrystalline spaces of sodium montmorillonite do not expand beyond a basal spacing of 19 Å. (ref. 7). The lower water content (0·62 c.c./gm.) for the heated montmorillonite indicates considerable but not complete loss of internal surface. The water content for 75 and 100 per cent lithium saturation is a little more than 0·4 c.c./gm. This water content indicates limited swelling between crystals since the initial porosity of the clay core was 0·11 c.c./gm.

In Fig. 2 the exchange capacity of the clay samples, used in the swelling work, is plotted against lithium percentage prior to heating. The exchange capacities determined by the method used by Greene-Kelly and by strontium saturating were in good agreement; the strontium content of the clay was determined by an X-ray spectrographic method. The exchange capacity (0·30 m.equiv./gm.) for the lithium-saturated clay was much higher than reported earlier. This exchange capacity decreased to 0·20 m.equiv./gm. when the clay was heated to 300° C. These values for the exchange capacity appear to be too large to be accounted for by exchange on the external surfaces

of the crystals since the crystals would have a surface area in the region of 50 m.²/gm.

It appears that about 75 per cent saturation with lithium and heating to 190° C. are able to prevent crystalline expansion. At 56 per cent lithium saturation there is some penetration into intracrystalline spaces but large crystalline swelling is not observed. At 25 per cent lithium saturation, montmorillonite exhibits large physical swelling as the result of expansion beyond a potential barrier at a silicate sheet separation of 19 Å.

It can be concluded that large crystalline swelling is not obtained at a surface density of charge of about $0{\cdot}6 \times 10^{-7}$ m.equiv./cm.², but it is obtained at a surface density of charge of 1×10^{-7} m.equiv./cm.².

We are indebted to Dr. K. Norrish for the determination of strontium.

J. P. Quirk
B. K. G. Theng

Department of Agricultural Chemistry,
Waite Agricultural Research Institute,
University of Adelaide.

[1] Hofmann, U., and Klemen, R., *Z. anorg. Chem.*, **62**, 95 (1950).
[2] Greene-Kelly, R., *Clay Minerals Bull.*, **2**, 52 (1953).
[3] Greene-Kelly, R., *Min. Mag.*, **30**, 604 (1955).
[4] Johns, W. D., and Tettenhorst, R. T., *Amer. Min.*, **44**, 894 (1959).
[5] Aylmore, L. A. G., and Quirk, J. P., *Nature*, **183**, 1752 (1959).
[6] Aylmore, L. A. G., and Quirk, J. P., National Clay Conference, Purdue University (1960) (in preparation).
[7] Norrish, K., and Quirk, J. P., *Nature*, **173**, 225 (1954).

MATHEMATICS

Theory of Optimal Games

Theory of games, and also theory of fair games, are well-known subjects, but a theory of good (or optimal) games does not seem to exist.

To theory of optimal games there would, for example, belong the question: Why, among serious poker-players, is it accepted as 'better' to play the game with a full pack and a hand of five cards rather than with, say, two Piquet packs shuffled together and a hand of seven cards? (The historical reason for this acceptance is not known, for the origin of poker is as obscure as is that of its name.)

That a game may possess different states of goodness is well seen from the varieties of two-handed whist. These may be epitomized thus: n full packs are shuffled together and equally divided among the two players; of the $26n$ cards that each player receives, kp are dealt face down to form k packets of p cards each; k are dealt face up, one on top of each packet, and the remaining $26n - k(p + 1)$ cards form the player's hand, from which, before the trump is declared, he must throw away r cards face down. When one of the k top cards is played, the card underneath it is turned up; and so on. In these games, obvious examples of zero goodness are (for all n), first, $k = r = 0$, and, secondly, $p = r = 0$, $k = 26n$; whereas, empirically, $n = 2$, $p = 3$, $k = r = 8$ 'makes rather a good game'.

In the context of theory of optimal games, it may be that croquet should receive consideration, for, in its (fairly short) history, it has, more than once, been felt non-optimal, and its controllers have altered the rules fundamentally (the most traumatic alterations being, perhaps, the decisions, first, to abandon play by rotation of colour, secondly, to award an optional lift against a player who is 'doing too well').

There would arise also the question: How far is competition a prerequisite of goodness? In patience games, this element is, naturally, lacking, yet many of the games are clearly 'very good', most notably, perhaps, those of the *Miss Milligan* series (of which Miss Milligan itself is, by trial and error, the optimal member). Croquet is a competitive game: the player of black-and-blue achieves success if he 'goes round' before the player of red-and-yellow; but there seems no reason why a 'croquet-patience', in which the two players combine to get all four balls round in as few turns as possible, should not be an equally good game.

Clearly, the great difficulty in theory of optimal games will be the establishment of a criterion, or measure, of goodness, and it may be that this establishment will not fall within the domain of mathematics; but, once a measure has been established, some mathematical analysis or, at least, a mathematical envisagement, of the problem might well be possible.

Alan S. C. Ross

University of Birmingham.

A Remark on Stochastic Path Integrals

Various stochastic functionals of the type:

$$S(t) = \int_0^t V(X(u), u)\, du \qquad (1)$$

where $X(u)$ is a stochastic process, have been studied in recent years (see, for example, Kac[1]). It seems worth noting that for $X(u)$ Markovian, there is often no particular difficulty in setting up characteristic function equations, of the type derived in my book[2] on stochastic processes (see especially the end of chapter 3), applicable not only to (1) but also to rather more general types of integral. Thus for:

$$S(t) = \lim_{\Delta u \to 0} \sum_{u=0}^{t} v(X(u), u, \Delta X(u), \Delta u) \qquad (2)$$

provided

$$E\,[|\{\exp(i\theta \Delta S + i\varphi \Delta X) - 1\}|\, X(t) = x] = \Psi_S(i\varphi, i\theta, t, x)\Delta t + o(\Delta t)$$

we may set up an operator equation of the form:

$$\frac{\partial C}{\partial t} = \Psi_S\left(i\varphi, i\theta, t, \frac{\partial}{\partial i\varphi}\right) C \qquad (3)$$

where $C \equiv E\,\{\exp(i\theta S(t) + i\varphi\, X(t))\}$. A particular case given on page 86 of my book is $v \equiv X(u)\Delta u$. As further examples, consider $v \equiv [\Delta X(u)]^2$, for (i) the normal linear Markov process (which includes 'Brownian motion' as a special case) and (ii) the simple birth-and-death process, for which $S(t)$ records the total number of transitions. For (i), $S(t)$ then increases regularly with t (as is well known); for (ii), C is readily evaluated, either by (3) or even more easily from the corresponding 'backward' equation. A further amplification of these points will be given elsewhere.

M. S. Bartlett

The University,
Manchester 13.
July 28.

[1] Kac, M., "Probability and Related Topics in Physical Sciences" (New York, 1959).
[2] Bartlett, M. S., "An Introduction to Stochastic Processes" (Cambridge, 1955).

FORTHCOMING EVENTS

Monday, September 12

BONE AND TOOTH SOCIETY (at 234 Great Portland Street, London, W.1), at 4.30 p.m.—Scientific Papers.

Saturday, September 17

BRITISH INTERPLANETARY SOCIETY (in the Tudor Room, Caxton Hall, London, S.W.1), at 6 p.m.—Mr. P. A. E. Stewart: "A Programme for the Surface Exploration of the Moon".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

BOTANIST or BIOCHEMIST, Scientific Officer grade (with an interest in field work), to grow and test plants for suitability as sources of protein—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (September 20).

LECTURER (with a particular interest in industrial organization or in advanced economic theory) IN ECONOMICS—The Registrar, King's College, Newcastle upon Tyne 1 (September 24).

SENIOR LECTURER IN SURGERY—The Registrar, The University, Sheffield (September 30).

LECTURER (with special qualifications or interests in cytogenetics or experimental taxonomy) IN BOTANY in St. Salvator's College—The Joint Clerk to the University Court, College Gate, St. Andrews (October 1).

LECTURER (with an honours degree, and preferably with a higher degree in chemistry with postgraduate experience of academic or industrial research) IN PHYSICAL CHEMISTRY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3 (October 1).

ASSISTANT LECTURER (with special qualifications in physical chemistry) IN CHEMISTRY—The Registrar, The University, Manchester 13 (October 15).

LECTURER (with an honours degree in psychology and some knowledge of statistical methods) IN PSYCHOLOGY IN THE DEPARTMENT OF EDUCATION—The Secretary, Cambridge University Department of Education, 17 Brookside, Cambridge (November 3).

ASSISTANT LECTURER IN PHYSICS—The Registrar, The University, Nottingham.

BIOCHEMIST or CHEMIST (preferably with some experience in protein or enzyme work or in the natural product field), for work of a fundamental nature on the chemistry and biochemistry of plant proteins—The Director, Arthur D. Little Research Institute, Inveresk, Midlothian, Scotland.

ENTOMOLOGIST (with a good honours degree in zoology or entomology, preferably with postgraduate research experience in forest or timber entomology) at the West African Timber Borer Research Unit, Kumasi, Ghana, to undertake studies of the biology, ecology and control of timber and lumber pests—Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.197/510/01/C1.

FISHERIES RESEARCH OFFICER, Senior Scientific Officer or Scientific Officer grade (with a good honours degree in zoology and at least two years suitable postgraduate research experience or training) with the East African Fisheries Research Organization, Jinja, Uganda, for research on ecology and fish physiology—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.195/194/02/C1.

GEOLOGISTS (with a first- or good second-class honours degree in geology with emphasis on metamorphic and igneous rocks and/or structural geology, some previous experience is desirable), for duties in Tanganyika including the regional geological survey of metamorphic and igneous rock terrains and preliminary investigation of mineral deposits—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.105/8/03/T.

HYDROLOGISTS (with a university degree in civil engineering and preferably with a postgraduate diploma in hydrology, with at least two years relevant experience; consideration would also be given to candidates with a degree in applied science with some direct experience in applied hydrology) with the Ministry of Works, Kenya, for duties including the measuring of river flows, assessment of flood discharges, rainfall run off studies and assessment of groundwater resources, with recharge and depletion—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.112/7/01/T.

LECTURER or JUNIOR LECTURER IN THE DEPARTMENT OF ZOOLOGY AND COMPARATIVE ANATOMY, to participate in the teaching of medical and postgraduate students and with good opportunity to undertake research work—The Secretary, St. Bartholomew's Hospital Medical College, West Smithfield, London, E.C.1.

PLANT PATHOLOGIST (with a good honours degree in a biological science and postgraduate experience in research or advisory work in plant pathology) in Tanganyika, for research and specialist advisory services in plant pathology—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/8/08/T.

RESEARCH ASSISTANT, for chemical and radiochemical analytical work on minerals—Dr. J. R. Butler, Geology Department, Imperial College of Science and Technology, London, S.W.7.

RESEARCH ASSISTANT (with an honours degree in physiology, physics or a biological subject), to assist in teaching and for experimental research in human lung function—Finance Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

RESEARCH ASSISTANTS (honours graduates in physics), for work on problems concerned with counting techniques and high energy physics, gas discharges, spectroscopy or electronics—The Clerk to the Governors, Northern Polytechnic, Holloway Road, London, N.7.

RESEARCH ASSOCIATE IN CHEMISTRY, for work on the physical chemical properties and synthesis of heteropoly compounds—Prof. Egon Matijevic, Department of Chemistry, Clarkson College of Technology, Potsdam, New York, U.S.A.

RESEARCH FELLOW (with qualifications in biochemistry), for work with Dr. P. T. Grant on the enzymic aspects of oxidase systems present in mammalian trypanosomes—The Secretary, University of Aberdeen, Marischal College, Aberdeen.

SENIOR SCIENTIFIC OFFICER or SCIENTIFIC OFFICER (with a first- or good second-class honours degree in zoology, and preferably some evidence of aptitude for original research) WITH THE EAST AFRICAN TRYPANOSOMIASIS RESEARCH ORGANIZATION, Tororo, Uganda, for work on the ecology of Glossina populations with special reference to sampling methods and to the factors determining population density and their relationship to modern methods—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.195/198/013.

LECTURER/SENIOR LECTURER (with at least an honours degree in mathematics, or equivalent qualification, and preferably with some research experience) IN THE SCHOOL OF MATHEMATICS, University of New South Wales, to lecture and undertake research in the field of pure mathematics and preferably in the field of modern algebra—The Agent General for New South Wales, 56–57 Strand, London, W.C.2 (October 7).

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletin of the British Museum (Natural History). Botany. Vol. 2, No. 7: The Identity of *Isopyrum aquilegioides* L. By Sir Gavin de Beer and William T. Stearn. Pp. 193–202. 6*s*. Entomology. Vol. 9, No. 3: Sphecidae (Hym.) Récoltés en Tripolitaine et en Cyrénaïque. Par Jacques de Beaumont. Pp. 219–251. 12*s*. Geology. Vol. 4, No. 5: A Review of the Upper Jurassic Pliosaurs. By Lambert Beverly Tarlo. Pp. 145–189+plates 20–28. 24*s*. (London: British Museum (Natural History), 1960.) [236

Ministry of Health. National Health Service. Electrical Safety in Hospitals—Electro Medical Apparatus. Pp. 2. (H.M. (60) 49). (London: Ministry of Health, 1960.) [236

Department of Scientific and Industrial Research and Fire Offices' Committee. Joint Fire Research Organization. Sponsored Fire-Resistance Tests on Structural Elements. By L. A. Ashton and P. M. T. Smart. Pp. xiii+84. (London: H.M. Stationery Office, 1960.) 7*s*. 6*d*. net. [236

British Association for Commercial and Industrial Education. A Guide to the Use of the Telephone in Business. Third edition. Pp. 20. (London: British Association for Commercial and Industrial Education, 1960.) 3*s*. net. [236

University of Leeds. Publications and Titles of Theses, 1957–58. Pp. 74. (Leeds: The University, 1960.) 2*s*. 6*d*. [236

Agricultural Research Council. A Bibliography of Farm Buildings Research, 1945–1958. Part 2: Buildings for Potato Storage. Pp. 31. (London: Agricultural Research Council, 1960.) 2*s*. [236

Lister Institute of Preventive Medicine. Report of the Governing Body, 1960. Pp. 33. (London: Lister Institute of Preventive Medicine, 1960.) [236

National Council for Technological Awards. Report for the period April 1959 to March 1960. Pp. 26. List No. 11: Diploma in Technology—List of 95 Courses (at 23 Colleges) which are now recognized by the Council as leading to the Diploma in Technology. Pp. 5. Memorandum on the Industrial Training of Students following Courses recognized as leading to the Diploma in Technology. Pp. 11. (London: National Council for Technological Awards, 1960.) [246

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1019, Vol. 252 (23 June 1960): A Contribution to the Structure of Coals from X-ray Diffraction Studies. By L. Cartz and P. B. Hirsch. Pp. 557–604. (London: Royal Society, 1960.) 15*s*. [246

The Beginnings of the Royal Society. By Margery Purver and Dr. E. J. Bowen. Pp. 16+2 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 2*s*. net. [276

Department of Scientific and Industrial Research. Moiré Fringes for Measurement and Control: a Booklet for the Machine-Tool and Engineering Industries. Prepared by the National Physical Laboratory and the National Engineering Laboratory. Pp. 11. (London: Department of Scientific and Industrial Research, 1960.) [276

The Ecology of Psychology: an Inaugural Lecture delivered on 3 February 1960. By Prof. George Seth. Pp. 20. (New Lecture Series No. 8). (Belfast: The Queen's University, 1960.) 2*s*. 6*d*. net. [276

Review of Coal Tar Technology, July–December 1959 (Vol. XI, Part 2). Pp. ii+85–161. (Gomersal, Leeds: Coal Tar Research Association, 1960). 10*s*. [276

Association of British Chemical Manufacturers. The Chemical Industry: a Career for You. Pp. 29. Appendix listing Chemical Firms offering opportunities for Careers. Pp. 39. (London: Association of British Chemical Manufacturers, 1960.) [276

Ministry of Power. The Nuclear Power Programme. Pp. 4. (Cmnd. 1083). (London: H.M. Stationery Office, 1960.) 4*d*. net. [286

Ministry of Power. Reports of H.M. Inspectors of Mines and Quarries under the Mines and Quarries Act, 1954, for 1959. North Western Division. By R. H. Clough. Pp. iii+29+4 plates. (London: H.M. Stationery Office, 1960.) 3*s*. net. [306

Electrothermal Engineering, Ltd. Resistors, Valve Retainers, and other Electronic Components. Pp. 22. (London: Electrothermal Engineering, Ltd., 1960.) [306

Courtaulds Limited. Report and Accounts, 1959–60. Pp. 30. (London: Courtaulds, Ltd., 1960.) [306

Ministry of Housing and Local Government: Department of Health for Scotland. Alkali &c., Works Regulations Act, 1906; Alkali, etc., Works Orders 1925–50; Alkali, etc., Works Regulations (Scotland) Acts, 1906 and 1951, and Alkali, etc., Works (Scotland) Orders, 1952 and 1959. Ninety-Sixth Annual Report on Alkali, etc., Works, by the Chief Inspectors, 1959. Pp. 69. (London: H.M. Stationery Office, 1960.) 4*s*. net. [306

Other Countries

Institut Royal Météorologique de Belgique. Publications, Série A, No. 12: Annuaire Climatologique pour 1958. Pp. 94. Observations Ionosphériques, Station de Dourbes. Novembre, 1958. Pp. 26. Decembre, 1958. Pp. 26. Janvier, 1959. Pp. 26. Février, 1959. Pp. 26. (Uccle-Bruxelles: Institut Royal Météorologique de Belgique, 1960.) [136

Colony of Fiji. Geological Survey Department—Annual Report for the year 1959. Pp. ii+21. (Council Paper No. 6 of 1960.) (Suva: Geological Survey Department, 1960.) 2s. [136

Institut Royal des Sciences Naturelles de Belgique. Mémoire No. 144: Ecologie d'Une Mare Oligotrophe et des Biotopes Contigus (Colonster, Province de Liége). Par Serge Jacquemart et Eugene Leloup. Pp. 50+3 planches. Mémoire No. 145: Essai d'Étude Statistique des Cyrtosprifer du Frasnien de la Belgique. Par Dr. Antoine Vandercammen. Pp. 175+5 planches. Deuxième Série, Fasc. 58: Recherches sur l'Aile des Chrysomeloidea (Coleoptera), Deuxième Partie. Par Dr. Pierre Jolivet. Pp. 152+planches 21–40. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1958 and 1959.) [136

Tea Research Institute of East Africa. Pamphlet No. 18: Elementary Notes on Tea Manufacture, Part 1: Withering. By Dr. R. Child. Pp. 16+4 plates. (Nairobi: Tea Research Institute of East Africa, 1960.) [146

Consejo Superior de Investigaciones Cientificas. Publicaciones del Instituto de Quimica "Alonso Barba", Vol. 13, Año 1959. Pp. ii+278. (Madrid: Instituto de Quimica "Alonso Barba", 1960.) [146

Geological Survey of Uganda. Report No. 2: Explanation of the Geology of Sheet 87 (Rakai). By Dr. W. J. Phillips. Pp. iv+48+2 plates+sheet 87. (Entebbe: Government Printer, 1959.) Shs. 15. [156

Centro Brasileiro de Pesquisas Fisicas. Notas de Fisica. Vol. 5, No. 18: Note on the Effects of Polarization of an Incident Photon Beam on the Azimuthal Angular Distribution of the (γ, p) Reaction Products. By A. G. de Pinho Filho. Pp. 257–269. Vol. 5, No. 19: Causality and Dispersion Relations for Fixed Momentum Transfer. By H. M. Nussenzveig. Pp. 273–307. (Rio de Janeiro: Centro Brasileiro de Pesquisas Fisicas, 1960.) [156

Republic of the Sudan. Annual Report of the Government Analyst for the year 1958–1959. Pp. ii+40. (Khartoum: Government Analyst, Wellcome Chemical Laboratories, Ministry of Health, 1960.) [226

Merentutkimuslaitoksen Julkaisu: Havsforskningsinstitutets Skrift. No. 185: Finnish Light-Ship Observations on Currents and Winds in 1955, 1956 and 1957. By Svante Nordström. Pp. 70. No. 186: The Baltic Cruise with *R/V Aranda* 1957—Hydrographical Data. By Ilmo Hela and Folke Koroleff. Pp. 16. No. 187: Die Eisverhältnisse in den Finnland Umgebenden Meeren im Wintern 1957–1958. Redigiert von Erkki Palosuo. Pp. 22. No. 188: Die Eisverhältnisse in den Finnland Umgebenden Meeren in den Wintern 1938–45 und die Baltischen Eiswochen in den Wintern 1938–39. Von Risto Jurva und Erkki Palosuo. Pp. 69. No. 189: Vedenkorkeusarvoja 1958: Vattenståndsvärden 1958. (English summary: Water Level Records for the year 1958). By Eugenie Lisitzin. Pp. 59. No. 190: The Frequency Distribution of the Sea-Level Heights along the Finnish Coast. By Eugenie Lisitzin. Pp. 37. No. 191: Jäätalvi 1958/59 a Suomen Merialueilla. (Ice Winter 1958/59 along the Finnish Coast). Pp. 18. (Helsinki: Merentutkimuslaitos, Havsforskningsinstitutet, 1958 and 1959.) [226

United States Department of Commerce: Weather Bureau. Technical Paper No. 29: Rainfall Intensity—Frequency Regime. Part 5: Great Lakes Region. Pp. iv+31. (Washington, D.C.: Government Printing Office, 1960.) 1.50 dollars. [226

Museum of Comparative Zoology at Harvard College. Annual Report of the Director, 1957–1958. Pp. 28. Breviora. No. 125 (May 27, 1960): The Luminous Organs of *Proctoporus* (Sauria, Reptilia)—a Re-evaluation. By Willard D. Roth and Carl Gans. Pp. 12. Bulletin of the Museum of Comparative Zoology at Harvard College. Vol. 122, No. 6: A Transcription of Darwin's First Notebook on "Transmutation of Species". Edited by Paul H. Barrett. Pp. 245–296. Vol. 122, No. 7: Skeleton and Musculature of the Thorax of *Gelastocoris oculatus* (Fabricius) (Hemiptera Heteroptera). By Margaret C. Parsons. Pp. 297–357. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1960.) [226

Berichte des Deutschen Wetterdienstes. Nr. 65 (Band 9): Beitrag zu einer Theorie Stationärer Antizyklonen. Von Hans Haarländer. Pp. 29. Nr. 66 (Band 9): Jahreskarten des Wärme- und Wasserhaushaltes der Ozeane. Von Fritz Albrecht. Pp. 19. (Offenbach a. M.: Selbstverlag des Deutschen Wetterdienstes, 1960.) [226

University of California Publications in Botany. Vol. 32, No. 3: A Morphological Study of Three South African Gigartinales. By Florence Ellen Hewitt. Pp. 195–234+plates 26–32. 75 cents. Vol. 30, No. 5: Morphological Aspects of Parasitism in the Dwarf Mistletoes (*Arceuthobium*). By Job Kuijt. Pp. 337–436+plates 34–48. 2 dollars. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1960.) [226

United States Department of Agriculture. Home and Garden Bulletin No. 67: Insects and Related Pests of House Plants—How to Control Them. Pp. 16. (Washington, D.C.: Government Printing Office, 1960.) 15 cents. [226

Publications de l'Institut National l'Étude Agronomique du Congo Belge. Série Scientifique. No. 82: Insectes Nuisibles aux Fruits et aux Graines. Par Karl E. Schedl. Pp. 133. 70 francs. No. 83: Hôtes Entomologiques du Bois. 1: Espèces Relevées à Yangambi. Par Prof. R. Mayné, avec la collaboration de Prof. C. Donis. Pp. 116. 125 francs. (Bruxelles: Institut National pour l'Étude Agronomique du Congo Belge, 1960.) [236

United States Department of Commerce: U.S. Coast and Geodetic Survey. Special Publication No. 254: Principles Underlying the Interpretation of Seismograms. Prepared originally by Frank Neumann. Revised (1959) edition. Pp. iii+41. (Washington, D.C.: Government Printing Office, 1960.) 40 cents. [236

Commonwealth Scientific and Industrial Research Organization, Australia. Soil Publication No. 15: The Measurement of the Ion-exchange Properties of Soils. By B. M. Tucker. Pp. 40. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1960.) [236

Tobacco Research Board of Rhodesia and Nyasaland. Bulletin No. 8: The Production of Turkish Tobacco. Pp. 28. (Salisbury: Tobacco Research Board of Rhodesia and Nyasaland, 1960.) [236

Transactions of the American Philosophical Society. New Series, Vol. 50, Part 5: The Dal Pozzo-Albani Drawings of Classical Antiquities in the British Museum. By Cornelius C. Vermeule, III. Pp. 76. (Philadelphia: American Philosophical Society, 1960.) 2 dollars [236

United States Department of the Interior: Fish and Wildlife Service. Fishery Bulletin No. 173: Systematics and Biology of the Gizzard Shad (*Dorosoma cepedianum*) and Related Fishes. By Robert Rush Miller. Pp. iv+371–392. (Washington, D.C.: Government Printing Office, 1960.) 25 cents. [236

United States Department of the Interior: Geological Survey. Geological Survey Topographic Instructions. Multiplex Plotter Procedures: Instructions in the Standard Operating Procedures for the Multiplex Plotter, including a description of Equipment. (Book 2—Mapping Procedures. Part SF—Stereocompilation Procedures. Chapter 3F4—Multiplex Plotter Procedures). Pp. viii+45. (Washington, D.C.: Government Printing Office, 1960.) 45 cents. [236

United States Department of Commerce: Coast and Geodetic Survey. Publication 60–2: Geodetic Operations in the United States and in other Areas through International Co-operation, January 1, 1957, to December 31, 1959. (Report to the International Association of Geodesy of the International Union of Geodesy and Geophysics, International Council of Scientific Unions.) Pp. vi+38. (Washington, D.C.: Government Printing Office, 1960.) [236

National Academy of Sciences—National Research Council. Science in Space: a Report by the Space Science Board. Chapter 2: The Nature of Gravitation. Pp. iv+21. 1 dollar. Chapter 3: The Earth. Pp. iv+36. 1 dollar. Chapter 4: The Moon. Pp. iv+10. 1 dollar. Chapter 5: The Planets. Pp. iv+13. 1 dollar. Chapter 7: Physics of Fields and Energetic Particles in Space. Pp. iv+58. 1 dollar. (Washington, D.C.: National Academy of Sciences—National Research Council, 1960.) [236

National Academy of Sciences—National Research Council. Nuclear Science Series. NAS–NS 3006: The Radiochemistry of Americium and Curium. By R. A. Penneman and T. K. Keenan. Pp. vi+62. 0.75 dollars. NAS–NS 3008: The Radiochemistry of Rhodium. By G. R. Choppin. Pp. vi+32. 0.50 dollars. NAS–NS 3009: The Radiochemistry of Molybdenum. By E. M. Scadden and N. E. Ballou. Pp. vi+38. 1.50 dollars. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1960.) [236

World Health Organization. Classification of Pharmaceutical Preparations: a Survey of Existing Legislation. Pp. 56. (Originally published in the *International Digest of Health Legislation*, 1960, 11, 4–56.) (Geneva: World Health Organization; London: H.M. Stationery Office, 1960.) 2 Swiss francs; 3s. 6d.; 0.60 dollars. [236

Annals of the New York Academy of Sciences. Vol. 85, Article 1: The Metabolism of Oral Tissues. By Philip Person and 48 other authors. Pp. 1–499. (New York: New York Academy of Sciences, 1960.) 4.50 dollars. [236

Smithsonian Institution: Bureau of American Ethnology. Bulletin 173: Anthropological Papers, Numbers 57–62. Pp. iii+498+81 plates. (Washington, D.C.: Government Printing Office, 1960.) [236

British Honduras. Annual Report of the Forest Department for the year 1958. Pp. 24. (Belize: Forest Department.) [236

National Academy of Sciences—National Research Council. Penetration of Charged Particles in Matter: Proceedings of an Informal Conference, Gatlinburg, Tennessee, September 15–18, 1958. (Nuclear Science Series, Report No. 29. Publication No. 752). Pp. x+174. (Washington, D.C.: National Academy of Sciences—National Research Council, 1960.) 2 dollars. [236

Mellon Institute Research 1959–1960: The Annual Report of the Chairman, Matthew B. Ridgway, to the Board of Trustees of the Institute, for the fiscal year ended February 29, 1960. Pp. vi+33. (Pittsburgh: Mellon Institute, 1960.) [236

New Zealand. Report of the Committee on New Zealand Universities, December 1959. Pp. 130. (Wellington: Government Printer, 1960.) [236

Tropical Fish Culture Research Institute, Malacca. Report for the two years 1957/8 and 1958/9. Pp. 15+5 plates. (Batu Berendam, Malacca: Tropical Fish Culture Research Institute, 1960.) [236

Ontario Research Foundation. Annual Report, 1959. Pp. 40. (Toronto: Ontario Research Foundation, 1960.) [246

Canada: Department of Mines and Technical Surveys. Annual Report Calendar Year 1958. Pp. vii+143. (Ottawa: Queen's Printer.) 1 dollar. [246

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada, Memoir 309: Permian Rocks and Faunas of Grinnell Peninsula, Arctic Archipelago. By P. Harker and R. Thorsteinsson. Pp. ix+89+25 plates. (Ottawa: Queen's Printer, 1960.) 2 dollars. [244

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.,
ST. MARTIN'S STREET, LONDON, W.C.2.
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £7/15/–, payable in advance, postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office